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QUALITY OF BEEF

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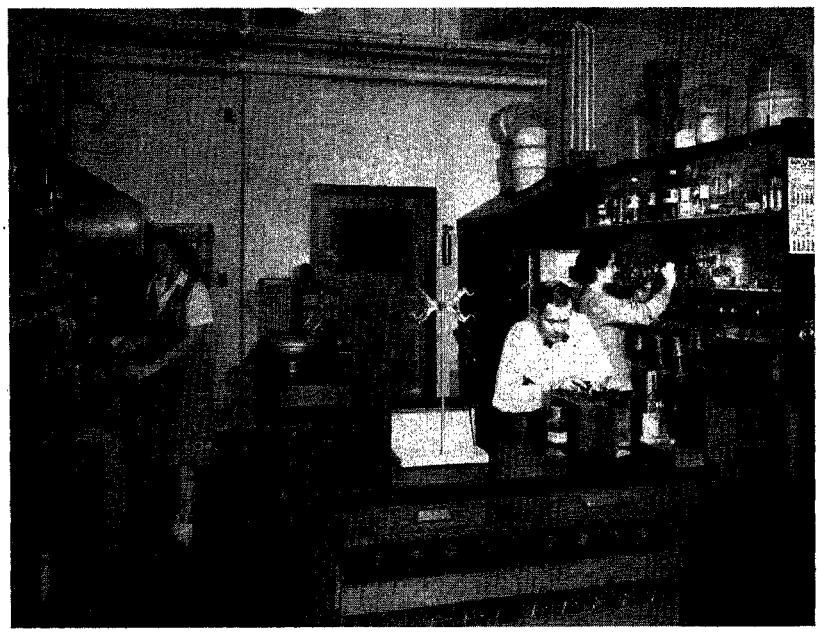

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QUALITY OF BEEF[1]

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J. L. HALL, C. E. LATSCHAR, and D. L. MACKINTOSH

SCOPE OF THE SUBJECT

Quality of beef is a subject of various implications to people involved in different phases of beef production and merchandizing. The livestock producer may use the term to imply his judgment of the condition of live beef animals as to fatness, conformation, and general well-being. The processor measures quality in terms of carcass characteristics, that is, fat covering, conformation, firmness, and color.

In retail markets the measure of quality is dominated by consumer reaction. It applies not only to visual characteristics, as color and distribution of fat and lean, but also to how the meat feels under the knife. A skillful meat cutter can make a fairly accurate prediction when he is preparing cuts for his showcase, as to probable tenderness of the meat after it is cooked. His honesty and reliability in this respect are extremely valuable assets in holding his trade. Tenderness seems to be the paramount quality desired in beef by the consumer.

In production and merchandizing usage, quality of beef implies its saleability. To the consumer it means eatability. Although tenderness appears to be the quality most insistently demanded by consumer trade, it need not be assumed that tenderness is the most important quality of beef. Rather, it may be that tenderness is one of the most variable qualities, and, likewise, one most likely to be hidden.

The consumer who buys beef not too skimpy in fat has a right to expect reasonable satisfaction in other palatability qualities, such

1. Contribution No. 284, Department of Chemistry; No. 156, Department of Animal Husbandry; and No. 120, Department of Home Economics.

as flavor, aroma, and juiciness. There are gradations in these latter qualities, but seldom to the extent of gradations in tenderness. Their relative real importance may be judged by the fact that the consumer quickly loses concern for all other qualities if the flavor happens actually to be disagreeable. Flavor is paramount.

Taint or off-flavor occasionally develops through poor keeping quality of beef in the coolers. Poor keeping quality also usually is attended by high shrinkage losses during storage or ripening. Attention of station personnel was called to reports of poor keeping quality of beef from animals grazed in a certain part of the state. Pasture soils in that area were suspected of being deficient in phosphorus. The investigations here reported were begun with the intention of examining those reports and the local conditions involved there.

The first part of that study included a broad investigation of the mineral elements in the blood and tissues of widely different grades of beef animals.

The second part included a comparison of keeping quality, shrinkage loss, and palatability of beef obtained from two lots of steers on different levels of phosphorus ration.

The third part was similar to the second part, in scope and procedure, but comparison was made between two lots of steers, one of which received limestone in its ration.

The fourth part was confined to color quality of lean beef. It applied primarily to dark-cutting beef, the ultimate purpose being to find its cause and control.

PART I. MINERAL CONSTITUENTS OF BLOOD, MUSCLE TISSUE, AND FAT TISSUE OF BEEF ANIMALS AND THEIR RELATION TO KEEPING QUALITY

INTRODUCTION

In view of the extent to which meat is used in the human dietary, surprisingly little information is available concerning the factors which may influence the various qualities of meat. Since Bancroft *(3)*[2] has shown that the character of an oil and water emulsion can be altered by changing the kind of emulsifying agent, and Clowes *(11)* has shown that the ratio of certain mineral elements in living tissue, especially sodium and calcium, has an effect upon its permeability, it might be expected that the mineral composition would bear a decided relation to shrinkage and keeping quality of meat. The effect of mineral balance on wetability of the walls of the intercellular spaces in tissues should in turn affect the capillary flow of intercellular fluid. That is, with an increasing ratio of monovalent, cation[3] to divalent cation it might be expected that the material would lose an increasing amount of moisture in storage; also, that the material would be more permeable to the action of bacteria, molds, and enzymes, since the mobility of these agents is greater in a water medium than in fat. *(5), (26), 30), (43).*

REVIEW OF LITERATURE

In an examination of the literature, limited data were found on the complete mineral analysis of the muscle tissues and adipose tissues. No information was found on the mineral analysis of muscle tissue and adipose tissue in relation to any quality considered in these investigations. Abundant data are available on the analysis of blood, but none of these was correlated with meat and its keeping quality.

Moulton *(35)* observed that the partial starvation of beef animals caused no increase in water content of the lean tissue but did cause a decrease in its nitrogen and phosphorus content when calculated to a fat-free basis. He also noted an increase in the water content and a decrease in the protein content of the blood. Moulton called attention to the importance of calculating data to a fat-free basis, and pointed out that erroneous conclusions may be drawn if the data are calculated to a water-free basis. Moulton *(36)* found also that the chemical composition of meat from mammals changed with age of the animal, a decrease in water content and an increase in ash and protein content being noted with increase in age.

2. Italic numbers in parentheses refer to Literature Cited, p. 83.

3. Cation. The ion which migrates toward the cathode during electrolysis of a solution of an electrolyte. In salts it is the basic element.

Ritchie, Moulton, Trowbridge, and Haigh *(45)* found that ages or levels of nutrition had little if any effect on the ash of the lean or ash of the fat of the rib when these were calculated to a fat-free basis. Neither did they have much effect on the total phosphorus in the round and rib as the level of nutrition was lowered. The total nitrogen in the lean flesh of the round, rib, and loin did not change noticeably with the condition of the animal but did increase as the animal increased in size.

Chatfield *(9)* found that the percentage of ash, protein, and fat varied with different cuts. The results of analyses for similar cuts from different animals were fairly uniform in most cases when these results were calculated to a fat-free basis as suggested by Moulton. There was, however, some variation in the results with degrees of fatness of the animal, even though the results were calculated to a fat-free basis.

EXPERIMENTAL

ANIMALS USED

Since the materials for analyses were available from the coöperative meat investigation project carried on at this station, it was decided to make an extensive analysis of the blood and of the muscle tissue and adipose tissues of a particular cut from all the project beef animals slaughtered in the fall of 1931. Eighteen animals were used.

Under the management of the Department of Animal Husbandry, 12 yearling Hereford steers, 17 to 18 months of age at time of slaughter, were fed alike during the winter of 1930-'31, and, May 1, 1931, were divided into three lots. Animals in lots 4, 5, and 6 came from other sources.

Lot 1.—Five head were full-fed ground corn, cottonseed meal, and alfalfa hay in the dry lot until September 29, when they were slaughtered.

Lot 2.—Five head were full-fed ground corn and cottonseed meal on bluestem pasture during this same period, and were slaughtered September 30.

Lot 3.—Two head were turned on bluestem grass until the time of slaughter, October 23.

Lot 4.—Two Shorthorn heifers about 18 months old at time of slaughter (September 28) had been on bluestem pasture (low in phosphorus) in another part of the state under private management and were the two best animals from that pasture. A number of other animals from the same pasture were showing signs of phosphorus deficiency, especially bone chewing.

Lot 5.—Two Hereford heifers (private management) about one year old at time of slaughter (September 28) had been on grass pasture similar to the one occupied by lot 4 and in the same region, but had received bone meal with their salt. None of the cattle from the lot 5 pasture was showing symptoms of phosphorus deficiency.

Lot 6.—Rib cuts (6th to 12th rib) from two animals commonly

known as dark cutters were obtained from Armour and Company in Omaha through the courtesy of W. J. Loeffel of the Department of Animal Husbandry, Nebraska Agricultural Experiment Station. Classification of animals according to feed is shown in Table 1.

TABLE 1.—FEEDING RATION OF BEEF ANIMALS FROM WHICH SAMPLES OF MEAT WERE TAKEN AFTER SLAUGHTER.

Lot No.	FEED	Sample No.
1	Full feed in dry lot (Manhattan)	1, 2, 3, 4, 5
2	Full feed on grass (Manhattan)	6, 7, 8, 9, 10
3	Pasture grass alone (adequate phosphorus, Manhattan)	11, 12
4	Pasture grass alone (low phosphorus)	13, 14
5	Pasture grass (low phosphorus) with bone supplement	15, 16
6	Feed unknown (dark cutters; only rib cuts obtained from Armour & Co., Omaha)	17, 18

METHOD OF PREPARING SAMPLES

Blood.—Blood samples, taken from the jugular vein, were obtained from all animals at the time of slaughter except those in lot 6. Glass jars in which the blood was collected contained sufficient ammonium citrate to serve as an anti-coagulant, and a few drops of a dilute solution of formaldehyde were added as a preservative, after which the samples were sealed and stored in the refrigerator until analyzed.

Muscle Tissue and Adipose Tissue.—The carcasses were dressed, split, and hung in the cooler at 2 C° for 120 hours. At the end of this period, the carcasses were cut up and from the left side the 9th- to 11th-rib cut was taken for chemical analysis.

The eye muscle, or rib eye (Longissimus Dorsi), other lean tissue, adipose tissue (a composite of all mechanically separable fat tissue), bone, and gristle were separated and weighed. (Table 2.)

The eye muscle was ground twice, first with a coarse grinder blade and then with a fine one. This procedure helped to avoid separation of connective tissue. Juice separating from the tissue was reincorporated with it after grinding.

The adipose tissue was ground in a refrigerated room (2° C.) to keep the tissue firm for effective cutting.

TABLE 2.—PHYSICAL ANALYSES OF RIB CUTS (9TH TO 11TH RIBS) EXPRESSED IN PERCENTAGE.

SAMPLE NUMBER	Eye muscle	Other lean	Bone	Adipose tissue	Gristle
1	22.02	29.00	12.55	34.90	1.53
2	17.43	39.88	12.04	29.25	1.40
3	20.84	36.82	13.34	27.85	1.15
4	21.87	33.56	12.74	30.43	1.40
5	18.96	32.35	10.71	36.62	1.34
6	24.87	32.48	13.04	28.00	1.61
7	22.33	35.26	12.21	28.53	1.67
8	21.38	34.98	12.61	29.58	1.45
9	21.89	33.68	13.66	29.52	1.25
10	21.33	34.73	12.91	29.39	1.64
11	23.85	40.32	17.57	16.29	1.97
12	25.00	41.55	18.48	11.41	3.56
13	21.75	36.25	17.47	20.81	3.72
14	18.72	38.68	16.83	22.92	2.85
15	17.81	34.46	18.07	28.32	1.34
16	16.58	34.03	18.01	28.73	2.65

Portions of the ground tissues for chemical analyses were weighed by difference from large weighing bottles to avoid evaporation. A short spatula for removing sample portions was included and weighed with 30 or 40 grams of the material in the weighing bottle. This quantity of material was ample for duplicate determinations of protein, ether extract, moisture, and ash.

Reserve sample material was stored in sealed fruit jars in a sharp freezer at—29° C.

TREATMENT OF MATERIAL FOR CHEMICAL ANALYSES

Whole Blood and Plasma.—The material was coagulated and partially dried in Vitreosil dishes in an air oven below 100° C. after which the temperature was raised slowly to about 150° C. This dried and faintly charred sample was placed on an electric heating unit and heated below dull redness while a low gas flame was applied carefully to the top surface. When charring was complete, the sample was removed to the muffle and ignited at a temperature just below dull redness (500° C.). Mineral constituents of whole blood are shown in Table 3. Mineral constituents of plasma from two representative samples from each of the first two lots are shown for comparison in Table 4.

Muscle Tissue (Longissimus Dorsi).—Samples were dried and ignited in the manner indicated for blood samples.

Adipose Tissue.—The material was dried in an air oven at 110° C., transferred to a hot plate (low heat), and burned with a small wick of ashless filter paper inserted in the melted fat. The charred residue was ignited at 500° C. in an electric muffle.

METHODS OF ANALYSIS

Calcium.—The ash from 100 ml. of blood or plasma, or from 100 g. of muscle tissue, was taken up in dilute HCl. The calcium was precipitated in the form of the oxalate from a dilute acetic acid solution as directed by Scott *(47)*, and titrated with N/50 $KMnO_4$.

The ash from 25 g. of adipose tissue was analyzed as directed in Methods of Analysis (1i), using N/50 $KMnO_4$.

Phosphorus.—The material (20 ml. blood or plasma, 2 g. muscle tissue, or 5 g. adipose tissue) was ashed with 10 ml. of 50-percent magnesium nitrate solution, with repeated small applications of concentrated to accomplish almost complete decomposition of organic matter before final ignition in the muffle at 500° C. Analysis was completed as directed in Methods of Analysis (1a).

OPTIONAL METHOD FOR CALCIUM AND PHOSPHORUS

Frequent loss of determinations occurred through explosive violence in oxidizing the material, especially adipose tissue, with magnesium nitrate. The following procedure was later adopted after demonstration of no loss of phosphorus by checking with above method.

Muscle Tissue.—A sample of 25 g. was placed in a 250-ml. beaker with 25 ml. water, 3 ml. concentrated H_2SO_4 and 25 ml. concentrated HNO_3 and heated on hot plate (low heat) until clear. Ten ml. of 60-percent perchloric acid was then added and heating continued until condensation of H_2SO_4 appeared on wall of beaker about 1 cm. above the bottom. If residue became darkened, it was cleared with a few drops of concentrated HNO_3 cooled, and made up to 100 ml. volume in a graduated flask.

An aliquot of 50 ml. was taken for calcium determination and evaporated to dryness in a 100-mi. beaker. Heating was continued with the cover glass removed until SO_3 fumes no longer appeared. The residue was taken up with 2 ml. of 20-percent HC1, warming if necessary to obtain clear solution. Five ml. of water, then 2.5 ml. of 5 normal ammonium chloride, 10 ml. of 2.5-percent oxalic acid, and a drop of indicator capable of registering pH 5 were all added. A mixture of methyl red and methylene blue was found satisfactory. A 1:1 dilution of strong ammonia was added until indicator turned green. Immediately more oxalic acid solution was added dropwise until indicator became pink (pH 5).

After standing over night the precipitate was filtered under suction with a fritted glass crucible and washed four times with 2-percent ammonia saturated with calcium oxalate. The filter crucible was returned to the 100-ml. beaker, 10 ml. of normal sulfuric acid added, and the oxalate titrated hot with N/50 permanganate. Since small amounts of calcium were found, it was necessary to carry through complete blanks containing exact amounts of reagents used, especially nitric acid.

For phosphorus determination, 1 ml. aliquot of the digest was transferred to a 100-ml. volumetric flask containing 50 to 60 ml. of water, and 1:3 ammonia was added until faintly pink to phenolphthalein. Ten ml. ammonium molybdate reagent (2.5 percent in 5 normal sulfuric acid) and 4 ml. aminonaphthol sulfonic acid reagent (0.5 g. in 195 ml. 15-percent $NaHSO_3$ and 5 ml. 20-percent Na_2SO_3 were added and made up to volume with water. The solu-

tion was compared colorimetrically with a standard solution made up in exactly the same way with 7 ml. standard phosphate containing 0.08 mg. phosphorus per ml. as described by Fiske and Subbarow *(14)*.

Adipose Tissue.—Samples of 10 g. each were placed in 250-ml. beakers and were dried over night in vacuum oven at 100° C., transferred to hot plate and burned with a small ashless filter paper wick. To the residue were added 25 ml. water, 3 ml. concentrated sulfuric acid and 25 ml. concentrated nitric acid. Digestion was completed as under muscle tissue.

For calcium determination 50-ml. aliquots were treated as under muscle tissue.

For phosphorus determination 5-ml. aliquots were neutralized in 50-ml. volumetric flasks with 1:3 ammonia and made barely acid to litmus with normal sulfuric acid. Five ml. of ammonium molybdate reagent and 2 ml. of aminonaphthol sulfonic acid reagent were added and made up to volume with water. The solution was compared colorimetrically with the nearest matching of a series of similarly prepared standards containing 1, 2, and 3 ml. standard phosphate with 0.08 mg. phosphorus per ml. (See reagents used under muscle tissue.)

Chlorine.—Chlorine was determined on the ash from 10 ml. of blood as directed in Methods of Analysis (1b).

Sodium and Potassium.—Sodium and potassium were determined as directed in Methods of Analysis (1c) upon the ash from 10 ml. blood or plasma, 5 g. muscle tissue, or 25 g. adipose tissue.

Magnesium.—Magnesium was determined on the filtrate following the separation of calcium as directed in Methods of Analysis (1d).

Moisture.—Two to 3 g. of the material was spread in a thin layer over the inner surface of a 7-cm. porcelain dish, placed in a vacuum oven, and partially dried at a pressure below 25 mm. of Hg and at a temperature of 60° C. for four hours. The dish containing the sample was then removed to a vacuum desiccator and dried to constant weight over concentrated sulfuric acid at a pressure less than 10 mm. of Hg, as directed in Methods of Analysis (1e).

Ash.—The material remaining from the determination of moisture was completely ignited in the muffle at a temperature just below dull redness, and the procedure in Methods of Analysis (1f) was followed.

Fat (Ether Extract).—Three to 5 g. of the material was spread in a thin layer over the surface of a thin sheet of fat-free absorbent cotton, then rolled, inserted into an extraction thimble which was placed in a 50-ml. beaker, and dried along with the portion for moisture determination. This dried material was then extracted 72 hours with anhydrous ether. Otherwise the procedure was that given in Methods of Analysis (1g).

Protein.—Approximately 2 g. of the material was rolled in a filter paper and dropped in an 800 ml. Kjeldahl flask; 18 g. of digestion mixture (HgO—80 g., $CuSO_4$—16 g., and K_2SO_4—1904 g.) and 37.5 ml. of concentrated H_2SO_4 were added. The material was digested for one hour after it became clear. It was then cooled and 400 ml. of water was added. Otherwise the procedure followed was the same as in Methods of Analysis (1h).

SHRINKAGE TESTS

The right side of each of the first 12 carcasses was stored in the cooler at 2° C. for approximately two weeks from the time of slaughter. At the end of this period the 6th- to 12th-rib cuts were removed and placed in the cooler again for the ripening test. The shrinkage of the rib cuts was determined for a storage period of 20 days.

DISCUSSION OF RESULTS

COMPOSITION OF BLOOD

A noticeable uniformity of substantial values for blood calcium is evident in the first lot in Table 3. These steers had alfalfa, a well

TABLE 3.—MINERAL CONSTITUENTS OF 100 ML. WHOLE BLOOD. WEIGHTS ARE GIVEN IN MILLIGRAMS.

SAMPLE NUMBER	Phosphorus	Calcium	Sodium	Potassium	Chlorine
1	18.72	9.88	267.44	50.83	297.47
2	20.32	9.60	276.49	51.40	304.73
3	17.05	8.80	283.65	38.36	264.82
4	22.10	9.69	269.64	37.13	268.45
5	25.00	9.59	256.51	71.04	250.32
6	20.20	8.27	278.71	51.40	277.52
7	24.07	9.97	272.65	48.19	281.15
8	24.07	7.67	263.54	58.17	302.91
9	18.67	7.82	257.94	53.80	270.26
10	23.44	10.74	241.31	58.75	253.94
11	21.06	7.81	275.28	32.83	233.99
12	23.03	7.55	269.64	34.17	214.04
13	21.78	9.87	270.61	43.64	301.10
14	18.88	8.53	268.08	50.24	288.40
15	13.99	8.28	250.00	65.84	273.89
16	18.88	10.31	256.59	52.64	290.22

recognized source of calcium. Consistently low values for calcium and potassium appear in lot 3. Deviations in this lot, however, should not be ascribed altogether to diet; these two animals had been regarded as culls and turned out on pasture at the beginning of the feeding experiment. Although the phosphorus averages for lots 4 and 5 from the two pastures deficient in phosphorus were below the averages of the other lots, the number of animals was too small to make the differences significant, especially since three of the 10 samples in the first two lots were practically as low in phosphorus.

TABLE 4.—MINERAL CONSTITUENTS OF 100 ML. PLASMA, EXPRESSED IN MILLIGRAMS.

SAMPLE NUMBER	Phosphorus	Calcium	Sodium	Potassium
4	15.14	12.47	323.49	23.56
5	17.64	12.32	343.23	31.85
9	16.60	11.90	328.19	15.84
10	15.82	13.79	341.37	24.92

Sodium was higher and potassium was lower in the plasma than in the whole blood as shown in Table 4.

COMPOSITION OF MUSCLE TISSUE

In view of the fact that the percentages of protein and ash were fairly constant in these samples of muscle tissue (Table 5), it followed that the sum of the percentages of ether extract and moisture should be nearly constant also. The average for the sums of ether extract plus moisture for this set of samples was 76.69 percent and the average deviation was 0.67 percent. The extreme deviation was 1.87 percent occurring in sample No. 7. Only two samples deviated more than 1.08 percent from the average. Since variations in ether extract and moisture in muscle tissue thus tended to compensate each other, analytical data stated as percentages of the whole muscle tissue were preferable to data reported on the fat-free basis, as shown by the constancy of phosphorus as a percentage of the whole muscle.

TABLE 5.—Constituents, Expressed in Percentage, of Whole Rib-eye Muscle (Longissimus Dorsi) From 9th- to 11th-Rib Cut Removed Five Days After Slaughter.

Sample Number	Water	Fat	Protein	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Ash
1	70.95	5.19	22.34	0.082	0.312	0.00536	0.0168	0.224	1.21
2	70.17	6.36	22.00	.081	.343	.00653	.0236	.222	1.12
3	73.01	4.08	21.38	.066	.324	.00603	.0258	.214	1.16
4	72.55	4.19	21.73	.070	.324	.00541	.0209	.209	1.13
5	71.11	6.37	21.73	.057	.303	.00580	.0247	.218	1.09
6	72.44	5.79	21.72	.056	.320	.00642	.0236	.214	1.07
7	72.65	2.17	23.56	.073	.323	.00635	.0280	.228	1.21
8	72.94	2.70	22.44	.073	.333	.00531	.0262	.226	1.14
9	70.64	6.08	21.47	.057	.355	.00629	.0236	.216	1.07
10	74.09	3.00	22.13	.051	.371	.00557	.0246	.229	1.15
11	75.09	1.78	22.25	.069	.361	.00546	.0247	.225	1.11
12	74.79	1.95	22.56	.069	.355	.00609	.0243	.220	1.12
13	74.28	1.33	22.78	.052	.370	.00682	.0263	.232	1.16
14	75.07	2.49	21.87	.065	.332	.00700	.0271	.209	1.08
15	75.34	0.71	22.53	.076	.363	.00988	.0282	.219	1.12
16	75.24	0.78	21.88	.068	.341	.00707	.0279	.218	1.10
17	72.63	5.11	22.44	.069	.323	.00390	.0237	.215	1.12
18	71.93	5.40	22.78	0.069	0.337	0.00539	0.0250	0.211	1.08

The data in Table 6 shows that a similar relation was found in other years.

TABLE 6.—CONSTANCY OF THE SUM OF ETHER EXTRACT AND MOISTURE IN BEEF-RIB EYE EXPRESSED IN PERCENTAGE.

YEAR	Number of animals	Ether extract plus water	Average deviation from mean
1930	8	76.89	0.24
1931	18	76.69	.67
1932	16	76.25	.41
1933	10	76.11	0.55

COMPOSITION OF ADIPOSE TISSUE

A dry, firm fat covering not only gives a carcass a desirable appearance but also is essential to good keeping quality and low shrinkage loss. The factors which appeared to be most intimately related to moisture content in beef adipose tissue were' (in decreasing order) phosphorus, protein, and the monovalent-to-divalent positive ion ratio (Table 7). The phosphorus alone follows the moisture so closely that moisture was closely approximated by the formula:

Percentage phosphorus x 322 = percentage moisture

An average deviation from the mean of 0.81 percent and extreme deviations of +2.01 and –1.87 percent were obtained. A lower mean deviation could be obtained by using all three factors mentioned above, but the extreme deviation was wider. The relation giving the lowest mean deviation found was:

Percentage protein/2 + (Na + K)/2 Ca + percentage phosphorus × 240 = percentage moisture.

The deviation from the mean was 0.70 percent moisture, and the extreme deviations were +2.01 and –2.05 percent. Sodium, potassium, and calcium were expressed in chemical equivalents. This same relation was applied to seven animals studied the previous year with an average deviation of 0.76 percent and extreme deviations of +1.46 and –1.36 percent. A similar relation has been found in other years, although the factor by which the percentage of phosphorus was multiplied varied apparently with the rainfall during six months previous to slaughter.

In fact the following empirical equation predicted the percentage of moisture in rib fat of steers slaughtered in September with an average deviation of less than 1 percent moisture from determined values over a period of 3 years (1930-'32):

$$\text{Percentage moisture} = \text{percentage protein} + \frac{\text{Wt. (NaCl + KCl)}}{\text{Wt. CaCl}_2} + \frac{RF}{0.16} \times \text{percentage phosphorus.}$$

RF represents inches of rainfall in the six months previous to slaughter, which occurred as follows: 1930, 24 in.; 1931, 22.5 in.; 1932, 15.8 in.

TABLE 7.—CONSTITUENTS, EXPRESSED IN PERCENTAGE, OF COMBINED MECHANICALLY SEPARABLE ADIPOSE TISSUE FROM 9TH- TO 11TH-RIB CUT, REMOVED FIVE DAYS AFTER SLAUGHTER.

SAMPLE NUMBER	Water	Fat	Protein	Sodium	Potassium	Calcium	Phosphorus	Ash
1	12.27	84.08	3.25	0.031	0.038	0.0255	0.037	0.22
2	11.97	84.44	3.59	.026	.035	.0228	.036	.24
3	12.19	84.59	3.43	.031	.040	.0219	.037	.22
4	10.90	86.53	2.84	.029	.034	.0202	.034	.31
5	10.45	87.23	2.56	.027	.032	.0178	.036	.23
6	10.69	85.89	3.97	.030	.033	.0197	.031	.20
7	11.74	84.70	3.40	.029	.030	.0210	.033	.20
8	10.80	85.92	2.91	.024	.034	.0222	.032	.26
9	11.51	85.19	3.40	.031	.037	.0273	.042	.30
10	11.04	85.11	2.93	.028	.036	.0237	.039	.28
11	14.34	79.14	6.56	.038	.052	.0277	.050	.29
12	16.89	74.79	8.17	.042	.060	.0301	.054	.33
13	11.40	84.17	4.22	.029	.033	.0240	.035	.24
14	15.39	80.48	4.78	.039	.042	.0317	.042	.27
15	17.10	76.60	5.53	.054	.054	.0271	.053	.34
16	12.87	81.48	4.19	0.034	0.047	0.0149	0.038	0.28

Since percentage composition of adipose tissue is affected greatly by relative size of fat cells, comparison of mineral balance can be shown better by ratios as in Table 8. The most evident abnormalities were the high Na/Ca and P/Ca ratios in lot 5. These animals were grass fed in an area deficient in phosphorus and were given a bone-meal supplement. Calcium may have been diverted from adipose tissue to bone structure when additional phosphorus was supplied. Relation of phosphorus and calcium is discussed in greater detail in Part II and Part III. Also noteworthy were the low P/N ratios in the lots on grass alone (lots 3, 4, and 5) as compared with the lots receiving corn (lots 1 and 2). These ratios indicated that even the so-called "adequate" pasture did not meet the full phosphorus requirement of the animals.

But probably of greatest significance was the low P/Ca ratios in lot 4, especially No. 14, in relation to shrinkage and keeping quality discussed under the following subtopic.

TABLE 8.—DISTRIBUTION OF MINERAL CONSTITUENTS OF ADIPOSE TISSUE. PERCENTAGE RATIOS FROM DATA IN TABLE 7.

SAMPLE NUMBER	K/Na	Na/Ca	P/N	P/Ca	Ca/N
1	1.22	1.22	0.071	1.45	0.049
2	1.03	1.49	.062	1.56	.040
3	1.29	1.42	.067	1.69	.040
4	1.17	1.44	.074	1.68	.045
5	1.18	1.52	.088	2.01	.043
6	1.10	1.52	.048	1.59	.031
7	1.03	1.38	.060	1.57	.039
8	1.42	1.08	.068	1.44	.048
9	1.19	1.14	.076	1.53	.050
10	1.29	1.16	.083	1.65	.051
11	1.37	1.37	.048	1.80	.026
12	1.43	1.40	.041	1.79	.023
13	1.14	1.21	.051	1.46	.036
14	1.08	1.23	.055	1.32	.041
15	1.00	1.99	.060	1.95	.031
16	1.38	2.28	0.055	2.55	0.022

RELATION OF COMPOSITION TO SHRINKAGE

Since corresponding rib cuts were ripened in all cases, they were practically the same shape and were considered as similar geometrical solids having the same average density. Since the surface areas of similar solids are proportional to the two-thirds power of the volume, $W^{2/3}$ (W = weight of the cut) may be taken as a factor proportional to the surface area of the cut. This factor for the series of cuts divided by the factor for the smallest cut gave what was called the "surface ratio," or a direct comparison of the surface area of each cut with that of the smallest cut. On the assumption that the loss in weight was proportional to the surface exposed, the weight lost was divided by the "surface ratio" to obtain the relative loss

per unit area as given in Table 9 (column 5) and Table 10 (column 6). These values represented the loss from a surface area equal to the surface area of the smallest cut.

TABLE 9.—SHRINKAGE LOSSES DURING RIPENING OF RIGHT RIB CUTS (6TH-12TH RIBS) STORED 20 DAYS AT 2° C.

SAMPLE NUMBER	Initial weight of cut	Shrink loss	Fraction of total water lost	Shrink loss per unit area*	Shrink loss per one percent H_2O content per unit area
	Grams	*Percent*	*Percent*	*Grams*	*Grams*
1	10,030	4.2	10.4	293	7.3
2	11,400	4.4	10.1	320	7.3
3	10,659	4.0	8.8	284	6.2
6	9,773	4.2	9.4	292	6.5
7	11,163	4.2	9.3	302	6.7
8	10,688	4.2	9.5	298	6.7
11	7,705	5.1	10.1	325	6.4
12	7,000	6.8	13.1	421	8.1
13	5,983	7.3	16.0	429	9.4
14	7,140	9.0	19.3	559	12.0
15	5,795	8.5	19.2	492	11.1
16	5,442	7.3	17.4	439	10.5

* Unit area = surface area of smallest cut.

A cut containing a relatively high percentage of water would naturally be expected to lose weight more rapidly during ripening than a cut containing a lower percentage of water, other factors being equal. On that assumption the loss per unit area was divided by the percentage of water content (disregarding bone) to obtain the factors in Table 9, column 6. These factors may be taken as a measure of permeability, since other variants, as size and water content, are compensated. These results showed the high permeability of the cuts from the animals on phosphorus-deficient grass.

In the case of the smaller cuts from the grass-fed animals it will be seen that the cuts from the two animals (samples 13 and 14, Table 9) receiving the low-phosphorus grass lost more weight during the ripening period than did the cuts from the two animals (samples 11 and 12) on pasture where there was no phosphorus deficiency. According to popular belief, the keeping quality of meat, as well as shrinkage during ripening, depends almost entirely on the amount of fat covering the cut. If this popular belief were true, then samples 13 and 14 from the animals receiving the low-phosphorus grass should have had higher keeping qualities than samples 11 and 12 from the animals on normal grass, for the latter samples contained only 16.28 percent and 11.41 percent, respectively, of mechanically separable adipose tissue; while the former contained 20.81 percent and 22.92 percent, respectively, of mechanically separable adipose tissue (Table 2). However, samples 11 and 12 were as free from spoilage than samples from full corn-fed animals and shrank only slightly more.

It is interesting to note that sample 14, which showed most spoilage during the ripening process, also showed the highest loss of water, and had the lowest P/Ca ratio in the adipose tissue. This high loss of water during ripening indicated that the water-holding power of this sample of meat differed from that of the other samples. Just what caused this loose combination of water and tissue was not determined. However, a deficiency of phospholipides in the tissue no doubt would materially reduce the quantity of water held by emulsification. Also the relatively high amount of calcium compared to phosphorus in the adipose tissue would reduce the hydrophyllic nature of the phospholipides.

The feeding of a limited quantity of bone meal seemed to have little effect on the structure of the meat produced, as samples 15 and 16 showed about the same keeping quality as did No. 13. Although samples 15 and 16 contained more fat (Table 2), they were of lower quality grade than samples 11 and 12 which were produced on normal grass. There appeared to be no direct correlation between the mineral composition of the samples, except the adipose tissue, and this loss of weight during cooking or ripening.

Cooking evaporation losses (Table 10) did not show so wide variation as ripening losses. The reason for the more constant cooking losses probably lies in the method of roasting. All roasts were brought to the same internal temperature, then removed from the oven. Therefore, a small roast would require less time in the oven and be subjected to less evaporation per unit surface area than

TABLE 10.—COOKING EVAPORATION LOSSES ON FRESH LEFT RIB ROAST (6TH-8TH RIBS).*

Sample No.	Weight roast before cooking	Total water in whole cut	Total cooking evaporation loss	Fraction of total water evaporated	Evaporation loss per unit area†	Evaporation loss per one percent H_2O content per unit area
	Grams	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*
1	4,728	40.5	7.3	18.0	173	4.3
2	4,856	43.7	7.5	17.2	181	4.1
3	5,033	45.5	8.5	18.7	206	4.5
4	5,570	43.4	8.1	18.7	202	4.7
5	4,794	40.2	7.9	19.7	189	4.7
6	4,205	44.5	7.6	17.1	174	3.9
7	4,815	45.1	9.2	20.4	220	4.9
8	4,469	44.3	9.3	21.0	219	4.9
9	4,624	42.6	8.5	19.9	200	4.7
10	4,508	44.8	7.8	17.4	182	4.1
11	3,456	50.5	8.6	17.0	184	3.6
12	3,683	51.7	9.6	18.6	210	4.1
13	1,820	45.5	8.2	18.0	142	3.1
14	2,068	46.6	10.7	22.9	194	4.2
15	1,683	44.2	7.7	18.2	130	2.9
16	1,941	41.8	9.0	21.6	159	3.8

* Methods of cooking and testing meat for palatability from Supplement to National Project Coöperative Meat Investigations, U. S. D. A. Cooking data were supplied through the courtesy of Martha M. Kramer of the Department of Home Economics, Kansas Agricultural Experiment Station.

† Unit area = surface area of smallest cut.

would a large one. Likewise a tissue relatively permeable to water would be relatively permeable to heat also (since water is a better heat conductor than fat), and the time required for roasting should be relatively short. Therefore, it seems that compensating factors tend to make cooking evaporation losses relatively constant as compared to ripening losses.

As indicated in the introduction, one of the reasons for undertaking this study was to determine whether the poor keeping quality of the meat produced by animals on an unbalanced mineral diet could be accounted for by an unbalanced mineral ratio in the meat. It was thought that the poor keeping quality might be due to an increase in permeability resulting from a high ratio of monovalent, to divalent minerals. The keeping quality of the meat from the animals on low-phosphorus grass was very poor.

Of the two samples obtained from the animals on low-phosphorus grass one, No. 14, spoiled during the ripening process so that it was unfit for consumption. The other sample, No. 13, from this lot was in very poor physical condition at the end of the ripening period. Sample 14, which spoiled during the ripening period, instead of having a high ratio of monovalent to divalent minerals, was actually next to the lowest of the 18 samples tested both in the muscle tissue and adipose tissue as shown below:

$$\text{Muscle tissue (percentage of } \frac{Na + K}{Ca + Mg} = 11.6)$$

$$\text{Adipose tissue (percentages of } \frac{Na + K}{Ca} = 2.56).$$

The sample showing the highest ratio of monovalent to divalent minerals in the muscle tissue (percentages of $\frac{Na + K}{Ca + Mg} = 17.8$) was No. 1, one of the steers full-fed in dry lot. This sample showed excellent keeping quality.

These results seem to indicate that there was little if any relation between the ratios of total monovalent to divalent minerals in muscle tissue and its keeping quality. A better relation was apparent with P/N, P/Ca, and Ca/N ratios in adipose tissue as shown in Table 8.

In this connection it should be remembered that the mineral analyses of these samples represented the total quantity of minerals only, and that no attempt was made to investigate the chemical and physical condition of these elements in the tissue. Furthermore, it seems likely that only the mineral elements absorbed in the surfaces of the intercellular spaces, or ionized in the intercellular fluid should bear any significant relationship to permeability of the tissue.

Studies have been begun on the relation of keeping quality in meat to ratios of minerals in various forms and conditions in the tissues. Wide differences have been found in mineral ratios in the expressible juice that could not be predicted from the ultimate analysis of the whole meat.

(For summary and conclusions, see page 79.)

PART II. EFFECT OF DIETARY PHOSPHORUS DEFICIENCY ON QUALITY OF BEEF[4]

INTRODUCTION

Although recently much attention has been given to phosphorus requirements in animal nutrition and to clinical aspects of phosphorus deficiency, little information appears available concerning the effects of phosphorus deficiency on tissues of the animal other than bone and blood. The purpose of this investigation was to observe the effect of phosphorus deficiency in beef cattle on the composition and quality (especially keeping quality) of muscle and adipose tissue.

In Part I were reported mineral analyses of muscle and adipose tissues from beef animals taken from an apparently phosphorus-deficient region in Kansas. It was reported that butchers in that region complained of the poor keeping quality of beef from animals taken from certain pastures, and refused to buy them on that account. Inspection of some of those pastures showed ample grazing, but many of the cattle were listless, had poor appetites, stood with their hind feet somewhat forward and with the back arched. They were reluctant to move even when closely approached by strangers. General poor condition was further indicated by rough, dry, shaggy hair coat. Similar symptoms were noted in experimental aphosphorosis by Riddell *(44)*.

Field tests on the soil of those pastures mostly indicated adequate phosphorus. However, a prevalence of soft, finely weathered limestone on the surface suggested doubtful availability of the phosphorus as plant food. This suspicion was confirmed by chemical analyses showing less than 0.1 percent phosphorus (dry basis) in the pasture grasses.

Another symptomatic evidence of phosphorus deficiency among the cattle was bone-chewing. A local butcher designated one pasture as the "bone-chewingest" pasture he had ever seen. The feeding of steamed bone meal was reported to have corrected the abnormal condition of the cattle, but the feeding of limestone appeared only to aggravate it.

Since results reported in Part I indicated poorer keeping quality and higher shrinkage losses in the beef obtained from that region, it was decided to induce phosphorus deficiency in steers under carefully controlled conditions at the Kansas Agricultural Experiment Station, and to compare the quality and composition of the carcasses with those from carefully matched controls.

4. Meat used in this investigation was obtained from steers used in the phosphorus-feeding project under the direction of J. S. Hughes, animal nutritionist, Department of Chemistry, and A. D. Weber, beef cattle specialist, Department of Animal Husbandry, Kansas Agricultural Experiment Station.

EXPERIMENTAL PROCEDURE

Three low-phosphorus feeding trials were conducted, one each in 1936, 1938, and 1940. Hereford steer calves, matched in pairs by weight as nearly as possible, were fed by the Mitchell *(33)* paired-feeding method. A ration was fed composed of pearl hominy, oats, beet pulp, blood meal, and prairie hay. Feed allotment for each pair was held to the level of the amount that the lower-consuming member would take. The ration provided six to seven grams of phosphorus per day per head. It was supplemented with monocalcium phosphate to provide a total of 20 g. of phosphorus per day for the high-phosphorus animal of each pair. Sufficient calcium carbonate was added to the ration to maintain a P/Ca ratio of 1:2 for each steer.

Since the third trial was the most successful in attaining typical phosphorus deficiency and in avoidance of complications, as later explained, detailed data for only that trial will be given and general comparison made with the other two trials.

In the first experiment, eight steers were used. Two pairs were slaughtered after 35 weeks and two pairs after 48 weeks of feeding. Although blood and bone analyses indicated successful induction of phosphorus deficiency in the low-phosphorus animals, two of the high-phosphorus animals were not satisfactory controls. One became highly excitable just before slaughter, and the other was the low-consuming steer of its pair, which could not be explained until a large abscess in the liver was discovered after slaughter.

In the second experiment 12 somewhat older calves were started. After 48 weeks of feeding, these animals did not develop low blood-phosphorus values characteristic of typical phosphorus deficiency on the low-phosphorus ration.

At slaughter the inorganic blood-phosphorus ranged from 5.2 to 8.9 mg. per 100 ml. serum in the low group; normal range is 8 to 10 mg. However, the bones of the low group had strikingly lower specific gravity and breaking strength. This condition seemed to indicate that the animals maintained their blood phosphorus requirement at the expense of the bones. Likewise, tissue phosphorus requirements evidently had been adequately maintained, since no distinct differentiation was evident, between the two lots in regard to phosphorus relationships in muscle and adipose tissues. Neither was any consistent difference in quality of the meat observed between the two lots.

In the third experiment 12 steers weighing from 344 to 393 pounds were started on the ration November 10, 1939, and were fed 44 weeks before slaughter. Although the members of each pair were on the same energy-intake level, the high-phosphorus animals gained from 50 to 140 pounds more than their pair mates with one exception; the low phosphorus animal of the first pair (the heaviest pair) gained nine pounds more than its mate. This pair was different in another noteworthy respect; although the inorganic blood phosphorus was

critically low in the low-phosphorus animal, the bone phosphorus and ash (Table 11) were notably high, almost as high as in its pair mate, which was highest of all the animals in that respect. Slaughter weight of the animals ranged from 568 to 779 pounds. Low-phosphorus steer No. 6 died from bloating. Data are given on the remaining five pairs.

After slaughter the carcasses were hung in the cooler five days before ribbing. The 6th- to 12th-rib cuts were removed from each side. The right rib cut was hung in the cooler for ripening and keeping quality observations. On each side the 12th-rib sections were removed for pH, oxidation potential, and color measurements. The 9th- to 11th-rib sections were used for physical and chemical analyses. The 6th- to 8th-rib sections were used as roasts for cooking and palatability tests.

RESULTS

Characteristics of phosphorus deficiency were evident in the composition of blood and bones of the low-phosphorus animals shown in Table 11.

The next, most obvious characteristic after low blood phosphorus was the higher blood phosphatase in the low-phosphorus animals of all but one pair. Unfortunately, phosphatase activity was not measured in muscle tissue. Later evidence (unpublished) has indicated higher phosphatase activity in dark-cutting beef than in bright beef. Further investigations on phosphatase activity in beef muscle tissue are in progress.

PALATABILITY

The left 6th- to 8th-rib cuts were roasted by standard procedure. Oven temperature was maintained at 300° F., and the roasts were removed when their internal temperature reached 137° F. An entire slice across the face of each roast was served hot to each judge. The samples were identified only by code numbers. Approximately six judges were selected from the college staff. As nearly as possible the panel of judges included the same personnel throughout the several years over which these investigations extended.

TABLE 11.—EFFECT OF HIGH- AND LOW-PHOSPHORUS FEEDING ON COMPOSITION OF BLOOD AT TIME OF SLAUGHTER AND COMPOSITION AND BREAKING STRENGTH OF BONES OF YEARLING STEERS.*

ANIMAL NUMBER	Blood analysis, 100 ml. serum			Bone analysis, 5th and 13th ribs (dry, fat-free basis)				
	Inorganic phosphorus	Phosphatase	Inorganic calcium	Ash	Phosphorus	Calcium	Breaking strength, 13th rib	Specific gravity, 13th rib
	Milligrams	*Units*	*Milligrams*	*Percent*	*Percent*	*Percent*	*Pounds*	
1 H†	8.2	6.1	11.30	62.57	11.73	23.45	410	1.40
2 L‡	3.0	15.5	13.50	61.22	11.07	23.24	140	1.18
3 H	8.3	5.9	11.60	60.96	11.19	22.92	300	1.34
4 L	3.3	11.3	11.44	56.63	9.92	21.84	115	1.17
7 H	6.8	6.7	11.30	59.39	11.11	22.41	310	1.33
8 L	3.7	15.0	13.01	51.43	9.18	19.72	200	1.07
9 H	7.3	9.0	11.20	59.22	10.79	22.31	470	1.34
10 L	2.9	21.1	12.30	54.83	9.86	20.91	160	1.02
11 H	10.8	13.3	13.10	59.96	11.08	22.51	360	1.26
12 L	3.5	11.4	11.80	55.99	9.98	21.17	470	1.18

* Analytical data taken from the phosphorus-feeding project by courtesy of J. S. Hughes and A. D. Weber.
† H-High phosphorus.
‡ L-Low phosphorus.

TABLE 12.—JUDGING COMMITTEE'S AVERAGE GRADES ON PALATABILITY FACTORS FOR BEEF RIB ROASTS FROM STEERS FED ON HIGH- AND LOW-PHOSPHORUS LEVELS.*

ANIMAL NUMBER	Tenderness	Flavor lean; desirability	Juiciness; quality	Aroma; desirability	Flavor fat; intensity	Texture
1 H	6.5	6.3	5.2	4.8	4.5	6.0
2 L	6.2	5.3	4.7	4.5	3.8	4.6
3 H	5.5	5.3	5.0	4.8	4.2	5.3
4 L	5.5	5.5	5.2	4.3	3.5	4.7
7 H	5.3	5.8	4.5	4.7	4.5	5.0
8 L	6.0	4.8	4.2	5.3	4.2	5.0
9 H	6.0	6.0	5.4	4.4	3.8	5.6
10 L	5.5	5.3	3.7	3.8	3.8	4.8
11 H	6.2	5.8	5.4	5.2	4.4	5.6
12 L	5.2	5.7	5.2	4.5	3.8	5.2

* Grade range: Lowest grade 1; highest grade 7.

It was evident that favor was shown the high-phosphorus roasts in all the palatability factors listed in Table 12. No more than one pair was judged in favor of the low-phosphorus roast under any one palatability factor. In three pairs the high-phosphorus roasts were given unanimous favor under all factors. In the other two pairs only two of the six factors favored the low-phosphorus roasts; in one of those pairs, Nos. 3 and 4, it is interesting to note that the carcass grade, Table 13, of the low-phosphorus animal was slightly higher than its pair mate in this one pair alone. In that same pair a slight advantage also appeared in the feeder grade of the low-phosphorus animal at the beginning of the experiment. In the first pair also a slight advantage in feeder grade went to the low-phosphorus animal, but the carcass grades were even. Further evidence of the advantage to carcass grade in the high-phosphorus animals is shown in the third and fourth pairs; here the feeder grades were even in both pairs, but a full grade difference appeared for the carcasses in the fourth pair.

Since carcass grade and estimation of meat quality depend a great deal on quantity and distribution of fat, mechanical separations were made of fat, lean, and bone in the 9th- to 11th-rib cut, as shown in Table 13. The most evident differential characteristic is the higher percentage of outside fat in the low-phosphorus cuts in all but one pair; in that one pair (the third) the fat covering was scanty in both cuts. Total fat was distinctly higher for the low-phosphorus cuts in the second and fifth pairs, and total fat was distinctly higher for the high-phosphorus cuts in the third and fourth pairs. In the first pair total fat was nearly even. Only fat distribution appeared affected by high and low levels of phosphorus feeding.

Although, as previously mentioned, the high-phosphorus animals made consistently greater gains in weight, it was evident that no parallel increased fat deposition occurred in them. Since bone and

TABLE 13.—FEEDER AND CARCASS GRADES, AND SLAUGHTER WEIGHTS IN POUNDS. PERCENTAGE COMPOSITION OF RIB CUTS FROM STEERS FED ON HIGH- AND LOW-LEVELS OF PHOSPHORUS.

ANIMAL NUMBER	Feeder grade*	Carcass grade*	Slaughter weight	Composition of rib cuts, 9th to 11th ribs				
				Rib eye	Other lean	Outside fat	Other fat	Bone and gristle
1 H	G	M+	704	22.6	37.0	4.9	16.5	19.0
2 L	G+	M+	714	23.5	38.5	6.9	14.9	16.2
3 H	G—	M—	779	23.0	38.5	3.9	13.9	20.7
4 L	G	M	662	22.7	36.4	5.9	19.2	15.8
7 H	G	M	655	26.7	36.4	2.8	16.1	18.0
8 L	G	CO+	622	28.6	36.2	1.8	14.2	19.2
9 H	G+	M	698	23.6	31.6	4.9	21.5	18.4
10 L	G+	CO	568	27.2	33.5	6.0	14.1	19.2
11 H	C—	G—	711	22.2	33.6	8.2	18.0	18.0
12 L	G+	M+	637	23.4	32.0	10.2	19.4	15.0

* Grade: C, choice; G, good; M, medium; CO, common. Grading charts Nos. 100, 101, and 102, Bureaus of Agricultural Economics and Animal Industry, United States Department of Agriculture.

muscle tissue contain a much higher percentage of phosphorus than fat tissue contains, they might be presumed to have been more restricted in their development by phosphorus deficiency than was the case with fat tissue. Conversely a higher percentage of the energy intake in the low-phosphorus animals might be assumed to have been converted to fat because of the restricted bone and muscle formation, thus accounting for the independence of fat formation from gains in body weight.

Such assumptions might be extended to explain higher palatability of high-phosphorus muscle tissue because of higher growth rate, the relation being analogous to the greater succulence and tenderness of more rapidly grown plant tissues. In support of that assumption may be cited the fact that four out of five animals weighing over 700 pounds (including No. 9) graded 6 or better in tenderness.

On the other hand, although the low-phosphorus animal, No. 2, gained nine pounds more than its pair mate, and the rib cuts from the two animals were nearly identical in physical composition, nevertheless the high-phosphorus roast graded higher in every palatability factor listed in Table 12, and especially high in flavor. The difference in quality was, therefore, evidently more deep-seated than would be apparent in routine physical grades and measurements. Similar palatability trends were found in the first two trials, though they were not so consistent, probably because of disturbing factors previously mentioned.

TABLE 14.—PERMEABILITY AS INDICATED BY COOKING TIME, KEEPING QUALITY, AND RIPENING SHRINKAGE LOSSES.

ANIMAL NO.	Cooking data		Keeping quality	Ripening shrink 28 days at 33° F.		
	Cooking time per pound	Evaporation loss per unit area*		Shrink loss	Shrink loss per unit area*	Shrink loss per one percent H_2O content per unit area*
	Minutes	*Grams*	*Order*†	*Percent*	*Grams*	*Grams*
1 H	23	141	3	9.0	601	12.3
2 L	26	121	3	9.1	606	12.1
3 H	24	153	2	8.4	568	11.5
4 L	25	129	2	8.0	526	11.3
7 H	28	163	3	8.7	583	11.4
8 L	29	166	4	12.5	755	13.9
9 H	26‡	144	2	7.7	504	11.4
10 L	28‡	123	4	11.5	699	13.5
11 H	23‡	145	1	6.6	449	9.9
12 L	33‡	152	1	7.4	492	10.9

* Unit area = surface area of smallest cut.

† Order of wholesome appearance and freedom from spoilage.

‡ Ripened samples. Data on fresh samples incomplete.

WEEPING QUALITY

The 6th- to 12th-rib cuts from the right sides were hung 28 days in a cooler at 33 ±1° F. They were then grouped and placed in order of keeping quality as judged by wholesome appearance and odor and freedom from spoilage. No observable advantage in keeping quality appeared for either member of three pairs, but in the other two pairs a distinct advantage appeared for the high-phosphorus cuts, as shown in Table 14.

Since no low-phosphorus cut exhibited better keeping quality than its pair mate and two pairs were definitely in favor of the high-phosphorus cuts, the net result is a strong indication of the better keeping quality of high-phosphorus beef. The number of samples was of course too small to be statistically significant.

In the first two trials of the experiment all cuts ripened satisfactorily with little spoilage and with no evident advantage for either high or low phosphorus.

The ability of a beef rib cut to endure ripening with minimum spoilage probably depends on three distinct qualities of the cut: First, the physical compactness and freedom from gaps between the muscles and fat deposits; second, ample fat coverage; third, low permeability of the tissues. Occasionally beef is flabby and tends to fall apart at the junction of tissue segments, making it susceptible to mold and bacteria penetration and large surface evaporation losses. This type of beef obviously is not suitable for ripening.

All the cuts used for ripening in this experiment were firm and compact.

As previously mentioned, fat coverage for each pair was more abundant on all but one of the low-phosphorus cuts, according to percentage outside fat shown in Table 13. However, in spite of this advantage there were higher ripening shrinkage losses (percent) for the low-phosphorus cuts in all but one of the pairs as shown in Table 14, column 5.

Shrinkage losses were adjusted for variation in surface area of the cuts (column 6) and again for total moisture content of fat and muscle tissues (column 7), as explained in Part 1. Surface area adjustment of losses still left only one high-phosphorus cut showing greater loss than its pair mate. It is interesting to note that this one (No. 3) was graded below its pair mate in two palatability factors and equal in another factor (Table 12). Also, it was the only high-phosphorus carcass with a grade inferior to its pair mate (Table 13), and the rib cut contained 7.3 percent less total fat than its pair mate. Strangely also it was the heaviest animal of all.

On the assumption that moisture losses were a function of the total water content of the cut (disregarding bone), other factors being equal, the loss per unit area was divided by the percent of total water content (disregarding bone). The resulting factor (Table 14, column 7) may be assumed to be a rough index of average permeability of the tissues. On this basis, the first two pairs indicated only slight and insignificantly higher losses (less than 2 percent more)

for the high-phosphorus cuts. The other three pairs indicate distinctly lower losses for the high-phosphorus cuts.

Furthermore, a striking relation was apparent between keeping quality and permeability by comparing the sequence of data in columns 4 and 7 in Table 14. Cuts ranking first in keeping quality ranged 9.9 to 10.9; second, 11.3 to 11.5; third, 11.4 to 12.3; fourth, 13.5 to 13.9. It was, therefore, evident that good keeping quality was closely indicated by low-permeability index in shrinkage losses.

Cooking data in Table 14 conform more with what might be expected from the relative amount of outside fat on the roasts as indicated by the composition of the adjacent cuts (9th to 11th ribs) in Table 13. The cooking time per pound was less in every case for the high-phosphorus roasts. Also in spite of the fact that these roasts were in the oven a shorter length of time, total evaporation losses were higher in three of the high-phosphorus roasts. In the third pair a slightly higher fat covering on No. 7 did not require a longer cooking time, but there was a slightly lower evaporation loss. Also in the fifth pair the change in order of evaporation losses from the trend in the other four pairs was probably caused by the excessively longer cooking time required by the low-phosphorus roast, exposing it to evaporation in the oven for a relatively much longer period of time than required by the high-phosphorus roast.

Because of the rapidly changing thermal conditions in the meat, changes in protein structure, and uneven periods of exposure to oven evaporation during cooking, such data were obviously not so satisfactory for comparing permeability as ripening shrinkage data, where uniform conditions were maintained for the same period of time for all cuts.

TABLE 15.—COMPARISON OF MECHANICAL SHEAR, COLLAGEN CONTENT, AND PALATABILITY GRADE FOR TENDERNESS ON RIB EYE OF FRESH AND RIPENED RIB ROASTS FROM STEERS FED ON HIGH AND LOW LEVELS OF PHOSPHORUS.

ANIMAL NUMBER	Mechanical shear on cooked sample		Collagen, fraction total protein		Palatibility tenderness	
	Fresh	Ripe	Fresh	Ripe	Fresh	Ripe
	Pounds		*Percent*		*Grade**	
1 H	13.8	13.5	5.15	4.98	6.5	6.7
2 L	16.8	18.4	5.23	5.77	6.2	6.0
3 H	15.4	17.6	7.52	8.67	5.5	5.0
4 L	13.3	14.6	6.20	5.12	5.5	5.8
7 H	16.9	14.4	6.18	7.24	5.3	5.8
8 L	13.4	14.8	5.59	6.10	6.0	6.5
9 H	16.2	14.9	6.01	6.02	6.0	6.2
10 L	16.2	14.7	5.87	6.58	5.5	5.8
11 H	15.0	15.4	5.09	5.64	6.2	6.3
12 L	17.5	17.0	7.00	5.73	5.2	5.5

* See Table 12.

TENDERNESS

Palatability grade for tenderness was in favor of only one low-phosphorus roast, but this roast was the only low-phosphorus sample receiving a higher aroma grade than its pair mate. Experienced judges are aware of the difficulty of differentiating palatability factors, even one which might seem so purely mechanical as tenderness. In view of the consistently higher palatability grades for high-phosphorus roasts in all factors shown in Table 12, some question might be raised regarding the independence of tenderness grades.

Comparisons of mechanical shear, collagen content, and palatability grades for tenderness are shown in Table 15. Mechanical shear was measured by the Warner-Brateler *(4)* machine, which records the force required to shear a core of meat, one inch in diameter. Collagen was determined by a modification of the Mitchell *(34)* water-extraction method. The ground meat and water were triturated 90 minutes in a pebble mill at 5° C. and washed through a 100-mesh sieve with warm water (50° C.). The residue was autoclaved and the gelatinized collagen was extracted with boiling water.

(Part III will give in more detail the method of protein-fraction separations).

In no case did mechanical shear indicate a pair tenderness relationship contradictory to palatability grade in the fresh samples. In the ripened samples contrary indications appeared in only two pairs where differences were small (less than 0.5 pound).

In only one pair, fresh or ripe, did more collagen appear in the sample judged more tender by eating. The judges found eight of the 10 samples more tender after ripening. The two found less tender also registered higher shear and contained more collagen in the ripened samples. However, six of the 10 ripened samples contained more collagen than the corresponding fresh samples. This anomalous condition has been noted frequently during the past 10 years of ripening studies at this station. It leads to a conjecture that the corresponding right- and left-rib cuts from the same beef carcass may not have been always so uniform before ripening as generally has been assumed.

In order to see if shrinkage losses during ripening could so reduce the moisture content of the rib eye removed from the ripened cut that an apparent increase in protein would occur, data obtained in 1933 are shown in Table 16.

As usual the rib-eye samples were taken from the 9th- to 11th-rib sections out of the 6th- to 12th-rib ripened cuts, and were therefore at least one inch from the exposed surface during ripening. It was obvious that no significant amount of moisture was lost from the rib eye which could account for a relative increase in the collagen of sample No. 221. Collagen in both fresh and ripened samples as shown in Table 15 was calculated as percent of total protein obtained in the fresh sample.

The high correlation of mechanical shear with collagen content and with palatability-tenderness grade was evident, in comparing

TABLE 16.—COMPARISON OF MOISTURE AND PROTEIN CONTENTS IN FRESH AND RIPENED RIB EYES FROM 9TH- TO 11TH-RIB CUTS RESPECTIVELY FROM THE LEFT AND RIGHT SIDES OF THE SAME CARCASSES.

ANIMAL NUMBER	Shrink 21 days	Moisture		Total protein		Collagen, fraction total protein	
		Fresh	Ripe	Fresh	Ripe	Fresh	Ripe
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
50.....	4.97	71.01	71.00	22.20	22.08	3.83	2.74
85.....	5.52	72.56	72.20	22.35	22.75	3.12	2.17
193.....	5.23	73.23	73.32	23.05	23.05	3.03	2.01
221.....	5.86	74.02	74.01	21.44	21.55	3.31	4.00
155.....	5.82	74.90	74.85	21.25	21.55	3.88	3.15

relative change in each sample during ripening. In this manner confusing differences in other palatability factors were eliminated as far as possible. In only two samples was the change in mechanical shear during ripening distinctly contrary in trend to the change in palatability tenderness grade during ripening. Likewise in only two samples was the change in mechanical shear distinctly contrary to change in collagen content during ripening.

It was thus evident that the Warner-Bratzler shear gave a reliable index of tenderness. In its use the authors have found special caution necessary to avoid mutilation with the cutter in order to remove a smooth, uniform core. The core cutter should be sharp; it should be rotated alternately in short strokes, and not continuously in the same direction; the entrance of the cutter should be directed after inspection of both faces of the roast in order to avoid large segments of connective tissue. Six to eight shear trials were made on one core.

JUICINESS

Palatability grade for quality of juiciness favored the high-phosphorus roasts in all pairs but the second, in which pair quantity also favored the low-phosphorus roast as shown in Table 17.

A higher grade for quantity of juice was given the high-phosphorus roasts in three pairs. But quantity of juice appears to be one of the most difficult palatability factors to differentiate from other factors, especially flavor, tenderness, and aroma. Unless judgment is quickly made, the grade may indicate quantity of saliva rather than quantity of juice from the meat.

It is reasonable to assume that quantity of juice judged by eating should correlate with quantity of fluid obtained from the meat with a mechanical press. No such correlation is evident in Table 17 with press fluid from either raw or cooked meat, but perfect correlation was apparent between quantity of press fluid and phosphorus level; greater quantity was obtained from the low-phosphorus sample in every pair, both raw and cooked. Results were similar in the first

TABLE 17.—RELATION OF JUICINESS TO PRESS FLUID AND COOKING LOSSES IN RIB ROASTS FROM STEERS FED ON HIGH AND LOW LEVELS OF PHOSPHORUS.

ANIMAL NUMBER	Palatability juiciness		Press fluid from 100 g. rib eye		Cooking losses	
	Quality	Quantity	Raw	Cooked	Drippings	Total
	Grade	*Grade*	*Ml.*	*Ml.*	*Percent*	*Percent*
1 H	5.2	6.0	48.5	21.5	1.56	7.79
2 L	4.7	5.8	52.0	28.2	1.32	7.13
3 H	5.0	5.3	51.0	24.0	1.53	8.60
4 L	5.2	6.0	54.0	32.7	1.83	7.90
7 H	4.5	5.7	56.4	24.7	1.13	8.84
8 L	4.2	5.2	58.5	29.5	0.95	9.08
9 H	5.4	5.2	51.0	19.1	2.63	9.56
10 L	3.7	5.7	54.2	28.0	1.24	7.79
11 H	5.4	5.8	51.2	6.8	3.56	10.24
12 L	5.2	5.2	57.0	28.5	4.22	12.16

year of the experiment, when phosphorus deficiency was successfully induced.

Such consistent behavior of press-fluid yields gives rise to strong suspicion that desirability of flavor and other palatability factors may frequently influence the reaction of the judges regarding quantity of juice.

Regardless of its doubtful relation with palatability grade for juiciness, press fluid provides valuable means of determining the relative degree of retention of various constituents of muscle tissue as shown in Table 18. In this case retention of calcium bore a significant relation to permeability of the muscle tissue. Higher yield of press fluid from the raw tissue further indicated the higher permeability of low-phosphorus beef previously discussed with keeping quality.

It was pointed out in Part I that calcium decreased permeability of muscle tissue. Therefore, it appeared significant that a higher yield of calcium was obtained in the press fluid of the low-phosphorus samples in each pair but the third, in spite of the fact that the corresponding original rib-eye tissues contained less total calcium than their pair mates. As a result, a higher retention of calcium in the high-phosphorus samples was clearly indicated.

Although calcium concentration in the press fluid was considerably below its concentration in the original tissue as shown in Table 18, phosphorus concentration was practically unchanged, indicating that it was not retained perceptibly by the pressed tissue. This relation was further illustrated by P/Ca ratios being generally higher in press fluid than in whole rib eye. Only about one-half the total phosphorus reacted as inorganic phosphorus by the Fiske and Subbarow *(14)* method for blood.

TABLE 18.—PHOSPHORUS AND CALCIUM IN 100 G. RIB EYE AND 100 ML. PRESS FLUID FROM RAW RIB EYE.

ANIMAL NUMBER	Total in rib eye			Rib-eye content			Total in press fluid		
	Phosphorus	Calcium	Ratio P/Ca	Ether extractable phosphorus	Water-soluble inorganic phosphorus		Phosphorus	Calcium	Ratio P/Ca
					Fresh	Ripe			
	Mg.	*Mg.*		*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
1 H	191	5.31	36.0	1.52	79	121	191	2.18	87
2 L	190	4.86	39.1	1.31	107	136	200	2.24	89
3 H	190	4.60	41.3	1.19	102	113	189	1.61	117
4 L	201	4.60	43.7	1.47	83	110	206	1.95	106
7 H	195	4.96	39.3	3.18	82	129	188	2.83	66
8 L	175	5.69	30.8	2.86	92	128	174	2.36	74
9 H	186	5.62	33.1	2.82	93	134	184	4.65	40
10 L	196	5.29	37.1	1.93	97	132	183	4.96	37
11 H	193	4.28	45.1	2.67	103	121	184	3.58	51
12 L	192	4.17	46.0	2.91	93	125	179	4.14	43

Press fluid was obtained by use of a Carver laboratory press (hydraulic) using a 2.25-inch test cylinder. In order to prevent extrusion of raw tissue past the plunger, 100 g. ground raw rib eye was mixed with three g. pulverized filter paper pulp (ashless) and stratified in the cylinder in 16 wafers separated by 5.5-cm. ashless filters. The felt pads were caulked outside with light strands of cotton fiber. The cylinder was placed in a tinned cup provided with a spout at the bottom so that the fluid could be drained directly into a graduated cylinder. For this purpose the cup was set upon a steel cylinder on the press platform to provide elevation for drainage. Minimum pressure to effect free flow of fluid was applied 15 minutes, when probably 90 percent of the yield was obtained. Pressure was gradually raised to 4,000 pounds per square inch in the next 15 minutes and maintained 15 minutes longer at that pressure.

Cooked tissue required no added paper pulp; 50 g. was divided in four portions between filters in the cylinder. Pressure was gradually raised to 4,000 pounds per square inch in 15 minutes, when the yield was taken.

The fluid and press cake were weighed, and from the weight of filter paper and meat used the loss (mostly evaporation) was found to be 4 to 6 percent. Considerable time was saved by using two cylinders, one of which was packed while the other was being pressed. The fluid was centrifuged before analysis.

When an ample supply of sample material is available, the Carver press method has the advantage of effecting a clean separation of plentiful amounts of fluid for analysis. The pressometer described by Child and Baldelli *(10)* has the advantage of being usable with small samples, two grams being sufficient.

There appeared to be a significant relation between quality grade of juiciness and yield of drippings in Table 17. In only the fifth pair, where quality-grade difference was small, was a lower grade given to the sample yielding more drippings. Also a significant negative correlation appeared between quantity grade of juiciness and total cooking losses. In only the first pair, where quantity grade difference was small, was a higher grade given to the sample yielding higher total cooking loss.

COLOR OF RIB EYE

As usual, the gross compositions of rib eyes (Table 19) were nearly uniform in protein and ash content. A small range of variation occurred between ether extract and moisture. The general occurrence of this relationship of rib-eye components was discussed in Part I.

Since marbling and degree of finish have been shown *(28)* to influence color grading of beef, it is interesting to note that ether extract of rib eye followed a better correlation with inter-muscular fat than with outside fat, as shown in Table 13.

TABLE 19.—COMPOSITION OF RIB EYE OF 9TH- TO 11TH-RIB CUT, EXPRESSED IN PERCENT.

ANIMAL NUMBER	Protein	Ether extract	Moisture	Ash	Total
1 H	21.40	0.95	75.00	1.20	98.55
2 L	21.35	1.10	75.65	1.10	99.20
3 H	21.60	1.03	75.27	1.07	98.97
4 L	21.55	2.38	73.40	1.09	98.42
7 H	21.50	1.00	75.20	1.10	98.80
8 L	20.05	0.66	77.65	1.03	99.39
9 H	21.00	3.08	74.15	1.06	99.29
10 L	21.00	0.70	76.20	1.16	99.06
11 H	21.50	2.10	75.33	1.04	99.97
12 L	20.60	2.55	75.55	1.08	99.78

Although color measurements indicated no tendency toward shady or dark beef in any of the samples, measurements were made on three of the most important factors related to color of beef muscle tissue as shown in Table 20.

(The significance of these factors will be discussed more fully in Part IV.)

TABLE 20.—HEMOGLOBIN IN BLOOD, MUSCLE HEMOGLOBIN, pH, AND OXIDATION POTENTIALS IN FRESH AND RIPENED RIB EYE.

ANIMAL NUMBER	Hemoglobin in blood	Muscle hemoglobin	pH measured by glass electrode		Oxidation potential ref. to H electrode	
			Fresh	Ripe	Fresh	Ripe
	Percent	*Percent*	*Units*	*Units*	*Volts*	*Volts*
1 H	9.90	0.44	5.50	5.52	0.126	0.100
2 L	11.41	0.45	5.70	5.70	0.131	0.103
3 H	10.83	0.35	5.61	5.60	0.144	0.081
4 L	10.71	0.42	5.44	5.57	0.127	0.112
7 H	11.18	0.39	5.20	5.65	0.106	0.092
8 L	9.21	0.42	5.62	5.65	0.096	0.100
9 H	12.12	0.36	5.42	5.65	0.117	0.122
10 L	.....	0.36	5.40	5.63	0.121	0.111
11 H	11.23	0.42	5.32	5.67	0.114	0.101
12 L	11.41	0.39	5.45	5.60	0.102	0.092

Muscle hemoglobin, which has been shown to have high correlation with both brilliance and chroma *(28)*, was slightly higher for the low-phosphorus sample in three of the five pairs, averaging 3 percent more for the low-phosphorus lot. The difference is not significant, and was similar to the results obtained in 1938. In 1936 the result was contrary with distinct differences. In conclusion, it was evident that no significant correlation existed between amount of muscle hemoglobin and dietary phosphorus level. Nor was any correlation evident between muscle hemoglobin and hemoglobin in the blood. Muscle hemoglobin was determined by spectrophotometric analysis described by Shenk, Hall and King *(48)*.

In only one pair was the pH distinctly more acid for the low-phosphorus sample. In the previous two years pH was measured by quinhydrone electrode, which was no doubt influenced by differences in oxidation potential. Comparison with third-year pH data was, therefore, not justifiable. Dark beef generally is less acid than bright beef, sometimes having a pH more than one unit higher than normal. Therefore, on that basis the low-phosphorus samples had a tendency toward the dark beef condition, which is most evident in sample No. 2. The glass electrode was inserted in a depression made by a large rounded glass rod in the 12th-rib slice; the tip of the calomel cell touched the surface of the slice. After ripening, eight of the samples had slightly higher pH values, all reaching approximately the same value near 5.65.

Oxidation potentials were slightly less positive for the low-phosphorus sample in three pairs and nearly even in the other two pairs. Dark beef has been found to have abnormally low oxidation potential, and a tendency toward that condition was indicated by the low-phosphorus samples again. It is noteworthy that the lowest potential occurred in a low-phosphorus sample, No. 8, and the highest potential occurred in a high-phosphorus sample, No. 3. After ripening, oxidation potentials decreased in eight samples, all reaching approximately the same value near 0.1 volt.

In the second trial, in which critically low values of blood phosphorus were not reached, both the highest and lowest oxidation potentials were found in high-phosphorus samples. In the pair in which greatest difference in potential occurred, the high-phosphorus sample had the higher potential. In other pairs differences in potential were relatively small.

In the first trial, oxidation potentials were all abnormally high on the fresh samples, suggesting some error in technic. But in the ripened samples the highest potential was found in a high-phosphorus sample; the lowest potential was found in a low-phosphorus sample: in three out of the four pairs the higher potential was found in the high-phosphorus samples, and in the fourth pair the potentials were almost identical.

It was, therefore, apparent from the three trials that a definite tendency toward low oxidation potential obtained in low-phosphorus beef muscle tissue.

Measurements of oxidation potentials were made with a Leeds and Northrup student type potentiometer between an inserted platinum rod and a calomel cell making contact with the tissue surface by means of the cell solution. Proper conditioning of the platinum electrode was found to be important in order to promote rapid equilibrium with the tissue. The two-inch lengths of No. 16 platinum wire were cleaned either in sulfuric acid-dichromate mixture or by ignition in a non-luminous gas flame. After being rinsed they were immersed in a 10-percent sodium bisulfite solution at least 10 minutes. They were thoroughly rinsed with distilled water before being inserted in the tissue. About 10 minutes was required to reach equi-

librium; this condition was evident when repeated potentiometer displacements of one millivolt alternately positive and negative from the balance point caused corresponding reversed deflections by the galvanometer. The galvanometer had a sensitivity of 0.125 microampere per division.

Another tendency toward a condition characteristic of dark beef was slightly evident in the higher inorganic phosphorus found in low-phosphorus samples in three pairs in Table 18. Again, No. 2 was outstanding in that respect as it was in pH as shown in Table 20, and the lowest inorganic phosphorus occurred in a high-phosphorus sample, No. 1. However, inorganic phosphorus in typical dark beef generally runs considerably higher than any of these values, 120 to 130 mg. per 100 g. Much higher values were found in the ripened samples in Table 18; apparently phosphoric acid was released from the hexose phosphates by hydrolysis during ripening.

CHARACTER OF FAT TISSUE

The undesirability of high moisture content in outside fat covering of beef was discussed in Part I. Also, the relation of phosphorus, protein, and mineral balance to moisture content was pointed out. Although the two outstanding high moisture values occurred in low-phosphorus samples as shown in Table 21, it is noteworthy that these samples contained the highest percentages of protein.

In four pairs the tissue concentrations of phosphorus as represented by P/N ratios were higher in the high-phosphorus samples. Results were similar in 1936. Calcium percentages were distinctly higher in the high-phosphorus samples in four pairs; results in the remaining pair (Nos. 9 and 10) were obscured by anomalous high values for calcium, phosphorus, and ash in No. 10, suggestive of contamination by bone chips or gristle.

Except in that same pair again, Ca/N ratios also were distinctly higher in the high-phosphorus samples, and P/Ca ratios were lower. Similar evidence of accelerated calcium mineralization of fat tissue in high-phosphorus steers was observed in all pairs in 1936; for, in spite of the fact that P/N ratios were higher in the high-phosphorus samples, the calcium was so much higher in those same samples that the P/Ca ratios were lower. It was thus evident that effects of phosphorus deficiency were more apparent in fat tissue than in muscle tissue.

(For summary and conclusions, see page 79.)

TABLE 21.—COMPOSITION OF OUTSIDE RIB-FAT TISSUE, AND CONTENT OF PHOSPHORUS AND CALCIUM IN 100 G. OUTSIDE RIB-FAT TISSUE.

ANIMAL NUMBER	Protein	Ether extract	Moisture	Ash	Phosphorus	Calcium	Ratio P/N	Ratio Ca/N	Ratio P/Ca
	Percent	*Percent*	*Percent*	*Percent*	*Mg.*	*Mg.*			
1 H	10.31	70.45	18.84	0.36	53.6	30.0	0.0325	0.0182	1.79
2 L	6.86	76.25	15.50	.29	44.1	12.8	.0400	.0116	3.45
3 H	8.94	72.82	17.20	.29	48.3	17.5	.0338	.0122	2.76
4 L	6.84	78.72	13.49	.22	36.5	10.0	.0332	.0091	3.65
7 H	11.24	71.20	18.55	.34	54.7	39.8	.0304	.0221	1.37
8 L	15.08	58.37	24.22	.37	55.3	17.4	.0234	.0072	3.18
9 H	6.72	81.42	11.78	.31	43.2	28.0	.0404	.0262	1.54
10 L	19.10	52.85	27.13	.70	107.0	96.2	.0351	.0315	1.11
11 H	5.33	81.90	12.55	.22	32.8	17.1	.0386	.0201	1.92
12 L	5.31	83.53	11.35	0.21	28.9	6.8	0.0340	0.0080	4.25

PART III. EFFECT OF FEEDING LIMESTONE SUPPLEMENTON QUALITY OF BEEF[5]

INTRODUCTION

Experiments conducted at the Kansas Agricultural Experiment Station (1920-1931) *(32)* demonstrated that the addition of ground limestone to certain cattle-fattening rations materially increased the efficiency with which cattle utilized the feed. A nutrition experiment was carried out at this station in 1937 and repeated in 1939 for the purpose of obtaining further specific information on the influence of ground limestone on a fattening ration for steers *(49)*. Meat from these steers was utilized in this investigation to determine what difference in quality could be observed as a result of feeding high and low levels of calcium.

In Part I of this publication, the role of mineral balance, involving calcium especially, was discussed showing that calcium tended to decrease permeability of animal tissue, thus improving keeping quality and decreasing shrinkage losses in beef.

Part II pointed out that low-phosphorus mineralization in beef muscle and fat tissues was accompanied by lower concentration and lower retention of calcium in the tissues. It was, therefore, undetermined whether deficiency of phosphorus or deficiency of calcium in the tissue, or perhaps a combination of both, caused inferiorquality in beef. It was believed further information on this question might be gained from the meat from steers fed on high and low levels of calcium.

EXPERIMENTAL PROCEDURE

Hereford steer calves, matched in weight and grade as nearly as possible, were fed by the Mitchell *(33)* paired-feeding method a ration of corn, cottonseed meal, and Atlas sorgo silage. Feed allotment for each pair was held to the level of all that the lower consuming member would take. To one member of each pair was given a supplement of 0.1 pound of ground limestone daily. For convenience the members of each pair were designated as high- and low-calcium steers.

The phosphorus intake of these steers was only slightly higher than the normal requirement. The addition of the limestone was sufficient to change the phosphorus-calcium ratio from 1:1 to 1:2, a more nearly optimum ratio.

Two trials of the experiment were conducted following the same procedure, one in 1937 with four pairs of steers and one in 1939 with six pairs. Since the second trial involved a larger number of animals, detailed data for that trial only are presented herewith; general comparisons are made with data of the first trial.

5. Meat used in this investigation was obtained from steers used in the limestone-feeding project under the direction of A. D. Weber, beef cattle specialist, Department of Animal Husbandry, and J. S. Hughes, animal nutritionist, Department of Chemistry, Kansas Agricultural Experiment Station.

In the first trial steers ranging in weight from 438 to 468 pounds each were fed 250 days and slaughtered in October. Final weights varied from 727 to 820 pounds. In two pairs the high-calcium steers gained more, and in two pairs they gained less than their pair mates.

In the second trial steers ranging from 342 to 401 pounds each were fed 190 days and slaughtered in June. Final weights varied from 625 to 715 pounds. In all six pairs the high-calcium steers gained 13 to 56 pounds more than the low-calcium steers.

The use of heavier steers in the first trial than those used in the second trial may have accounted for the difference in response to mineral deficiency. A similar experience was encountered in the experiment on phosphorus deficiency reported in Part II in which large steers on low-phosphorus ration did not develop critically low blood-phosphorus concentrations characteristic of extreme phosphorus deficiency.

After slaughter, carcasses were treated as described in Part II which described also the meat cuts taken for experimental use.

RESULTS

CALCICM AND PHOSPHORUS BALANCES

Only slight variation was observed in calcium and phosphorus in the blood. Blood calcium, surprisingly, was fractionally lower in the high-calcium steers in four pairs. Phosphorus showed a complementary effect to the calcium, being slightly higher for the high-calcium steers in five pairs as shown in Table 22.

TABLE 22.—EFFECT OF HIGH- AND LOW-CALCIUM FEEDING ON COMPOSITION OF BLOOD AT TIME OF SLAUGHTER AND COMPOSITION OF BONES OF YEARLING STEERS.*

ANIMAL No.	Blood analysis, 100 ml. serum		Bone analysis, 5th and 13th ribs (dry, fat-free basis)			Breaking strength, 13th rib	Specific gravity, 13th rib
	Inorganic phosphorus	Inorganic calcium	Ash	Phosphorus	Calcium		
	Milligrams	*Milligrams*	*Percent*	*Percent*	*Percent*	*Pounds*	
1 H†	6.4	10.1	56.71	10.65	20.88	270	1.288
2 L‡	6.8	10.9	56.58	10.72	20.93	240	1.260
3 H	8.3	10.1	57.83	10.98	21.66	430	1.431
4 L	6.0	11.0	55.18	10.34	20.38	230	1.250
5 H	7.9	11.8	58.97	11.06	21.94	215	1.450
6 L	7.7	11.7	57.24	10.67	21.29	130	1.282
8 H	7.7	11.0	58.49	11.06	21.68	350	1.440
7 L	7.5	11.4	54.81	10.49	20.80	120	1.182
10 H	8.5	12.1	59.11	11.10	20.66	260	1.393
9 L	8.2	12.1	55.83	10.63	20.51	175	1.276
12 H	8.7	11.4	58.20	10.96	21.67	290	1.422
11 L	7.7	11.7	53.74	10.49	19.98	135	1.239

* Analytical data were taken from the limestone-feeding project by courtesy of J. S. Hughes and A. D. Weber.
† High calcium.
‡ Low calcium.

Bone composition showed a distinct correlation with calcium intake in Table 22. Except in the first pair, in which the analyses were practically identical, bone ash, phosphorus, and calcium were consistently higher for the high-calcium steers. Distinctly higher values for breaking strength and specific gravity of the bones were also obtained for the high-calcium steers. It was evident, therefore, that steers on the lower calcium-intake were not receiving adequate calcium for optimum bone development.

Similar effects on bone mineralization were observed in the 1937 trial, indicating that the calcium deficiency was clearly evident in the bones before its effect could be noted in blood or tissues. Likewise in the phosphorus-feeding experiment previously mentioned, bones of the larger steers showed mineralization deficiency although phosphorus deficiency was not evident in blood or tissues.

PALATABILITY

Rib roasts were prepared and judged on palatability factors as described in Part II. In Table 23 are shown the judging grades on the same factors in which high-phosphorus beef showed superiority in the previous experiment.

TABLE 23.—JUDGING COMMITTEE'S AVERAGE GRADES ON PALATABILITY FACTORS FOR BEEF RIB ROASTS FROM STEERS FED ON HIGH- AND LOW-CALCIUM LEVELS.*

ANIMAL NUMBER	Tenderness	Flavor lean; desirability	Juiciness; quality	Aroma; desirability	Flavor fat; intensity	Texture
1 H	5.8	5.0	5.6	3.8	3.4	5.6
2 L	5.6	5.0	4.6	4.2	3.0	5.6
3 H	5.0	5.4	5.2	5.2	4.2	4.8
4 L	5.2	5.8	4.6	4.4	4.0	5.2
5 H	6.4	6.0	5.2	4.8	4.2	5.2
6 L	5.8	5.8	4.8	5.0	4.0	5.8
8 H	5.4	5.6	3.8	5.0	3.0	5.6
7 L	6.4	6.0	4.6	5.0	4.2	5.6
10 H	5.0	5.4	4.4	5.2	4.0	5.4
9 L	5.2	5.4	4.8	4.4	3.4	5.4
12 H	5.0	5.2	4.6	5.0	3.8	5.2
11 L	5.2	5.5	4.6	4.8	4.4	4.7

* Grade range: Lowest grade, 1; highest grade, 7.

No such clear distinction is evident in the palatability of high- and low-calcium beef. The number of pairs out of a total of six pairs in which the high-calcium sample drew a superior grade over its pair mate were as follows: tenderness, 2; flavor of lean, desirability, 1; juiciness, quality, 3; aroma, desirability, 3; flavor of fat, intensity, 4; texture, 1. In only one factor, intensity of fat flavor, were high-calcium samples superior in a majority of the pairs. However, low-calcium samples were superior in a majority of pairs under only one palatability factor, tenderness. Both were bare majorities,

TABLE 24.—FEEDER, CARCASS, AND SLAUGHTER GRADES, SLAUGHTER WEIGHTS IN POUNDS, AND PERCENTAGE COMPOSITION OF RIB CUTS FROM STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NUMBER	Feeder grade*	Carcass grade	Slaughter grade	Slaughter weight	Percentage composition of rib cuts, 9th to 11th ribs				
					Rib eye	Other lean	Outside fat	Other fat	Bone and gristle
1 H	11.3	21.5	18.6	654	26.4	40.3	.....†	14.7	18.5
2 L	11.3	22.5	19.3	660	26.0	40.4	.....	17.3	16.4
3 H	10.0	16.0	16.0	710	23.5	37.5	7.2	14.5	17.3
4 L	12.6	17.0	15.3	615	23.3	35.6	9.1	14.9	17.1
5 H	15.3	15.0	14.0	690	23.6	34.4	8.3	19.5	14.3
6 L	10.0	15.5	16.6	680	25.6	33.8	6.0	17.1	17.5
8 H	15.5	24.5	16.6	685	24.9	39.7	2.7	11.9	20.7
7 L	14.7	20.0	20.0	665	25.9	36.8	4.9	18.6	13.9
10 H	16.0	18.0	14.0	740	23.8	35.9	6.1	17.8	16.4
9 L	15.3	21.5	24.0	690	23.2	40.7	3.1	13.4	19.5
12 H	12.6	15.5	14.0	700	23.8	35.5	7.0	16.7	17.0
11 L	14.6	14.5	19.3	700	25.2	32.5	7.5	17.6	17.2

* 2-6, prime; 8-12, choice; 14-18, good; 20-24, medium; etc.
† Not weighed separately.

4 out of 6, and have little or no significance. The judging record therefore indicated practically no preference for either high- or low-calcium beef in regard to palatability.

However, a similar trend in tenderness was observed in the first trial, when only one high-calcium sample out of four was judged more tender than its pair mate.

Advantage in quality of the high-calcium steers is evident in Table 24. Although feeder grades were balanced almost evenly at the beginning of the experiment, slaughter grades were higher for the high-calcium steers in five pairs and were nearly identical in the remaining pair. However, carcass grades did not show as distinct classification, indicating higher quality for high-calcium carcasses in only four pairs and only by narrow margins. It is interesting to note the reversal in order from slaughter grade to carcass grade in the fourth and sixth pairs. The wide difference in fatness in the fourth pair, as indicated in the composition of the rib cuts in Table 24, undoubtedly influenced the carcass grades.

The amount of outside fat on the rib cuts appeared evenly balanced between the high- and low-calcium steers. However, in four pairs there was more total fat in the low-calcium cuts. In the phosphorus experiments more outside fat appeared on the low-phosphorus cuts, but total fat was balanced, even though high-phosphorus steers made better gains. Related effects on palatability were also discussed. Likewise even though high-calcium steers made better gains, it was evident that no parallel increased fat deposition occurred in them, as indicated by the rib cuts.

KEEPING QUALITY

The 6th- to 12th-rib cuts from the right sides were hung 21 days in a cooler at 33 ±1° F. All cuts ripened in good condition and differences in pair mates were not pronounced. However, they were placed in order of keeping quality as judged by clean, wholesome appearance and freedom from spoilage as shown in Table 25. Although order grades represented but small differences, the high-calcium cuts received higher rank in every pair.

In the fourth pair greatest difference appeared, and it is interesting to note that this low-calcium cut (No. 7) had considerably more fat, both outside and total, than its pair mate. It appeared, therefore, that the high-calcium rib cuts possessed better keeping quality than the ones on low calcium, regardless of fat coverage. Similar observations were previously reported for high-phosphorus rib cuts.

Although keeping quality appeared largely independent from amount of fat coverage, the same was not true of shrinkage losses. In the phosphorus experiments even the shrinkage losses appeared independent from amount of fat coverage (Part II). But as shown in Table 24 and Table 25 (column 5) in the five pairs on which fat data were complete, only two of the high-calcium cuts suffered a lower percent shrinkage loss than their pair mates, and in every pair a higher percent shrinkage loss was accompanied by a lower outside fat content.

TABLE 25.—PERMEABILITY AS INDICATED BY COOKING TIME, KEEPING QUALITY, AND RIPENING SHRINKAGE LOSSES.

ANIMAL NO.	Cooking data		Ripening shrink, 21 days at 33° F.			
	Cooking time per pound	Evaporation loss per unit area*	Keeping quality	Shrink loss	Shrink loss per unit area*	Shrink loss per one percent H_2O content per unit area*
	Minutes	*Grams*	*Order†*	*Percent*	*Grams*	*Grams*
1 H	25.5	208	2	11.7	784	15.3
2 L	22.0	289	3	11.1	770	14.7
3 H	23.2	318	1	9.4	700	14.6
4 L	26.9	290	2	9.4	674	14.5
5 H	25.3	285	1	8.1	600	13.0
6 L	23.7	374	2	9.5	672	14.5
8 H	22.2	329	3	10.6	748	14.4
7 L	23.8	267	5	9.5	693	13.9
10 H	23.2	420	3	9.1	665	13.8
9 L	26.1	286	4	10.6	743	14.6
12 H	23.8	335	3	9.5	700	15.0
11 L	25.3	319	4	8.5	620	13.5

* Unit area = surface area of smallest cut.
† Order of wholesome appearance and odor.

Even after shrinkage losses shown in Table 25 were adjusted for variation in surface area (column 6) and again for total moisture content (column 7), as described in Part I, higher losses were invariably associated with lower outside fat content. A similar correlation occurred in the first pair with total fat, where outside fat was not weighed separately. It, therefore, seemed evident that high calcium was not successful in reducing shrinkage losses in beef rib cuts. Furthermore, it was indicated that good keeping quality was not necessarily associated with low shrinkage losses when the same phosphorus level was maintained.

In the first trial of the experiment, high-calcium rib cuts suffered slightly higher ripening shrinkage losses than their pair mates in two out of four pairs. Although outside fat tissue was not weighed separately in the first trial, the higher shrinkage losses accompanied lower total fat tissue of the rib cuts. This relation was consistently displayed by both trials, and it indicated that shrinkage losses were more dependent on fat coverage than calcium feeding. These results were in contrast with the phosphorus-feeding experiments as previously mentioned.

Keeping-quality observations in the first trial indicated that the rib cut ripening in the poorest condition was in the low-calcium lot; the one in the best condition was in the high-calcium lot. No distinct difference appeared in the others. Consequently the high-calcium cuts appeared to have slightly better keeping quality.

The cooking time per pound was less for four of the high-calcium roasts, but the corresponding cooking evaporation losses were

greater, as shown in Table 25. It was significant that three of those four samples had less outside fat than their pair mates as shown in Table 24, accounting for the more rapid penetration of heat and the more rapid escape of water vapor. Such simple explanation did not apply to the fifth pair, however; in spite of the lower amount of outside fat tissue and total fat tissue in No. 9, it required longer cooking time per pound and yielded one-third less evaporation loss than No. 10.

However, the ripening shrinkage losses in this pair were opposite in trend to the cooking evaporation losses, whereas, in four of the six pairs, these two losses followed the same trend. It, therefore, seemed evident that an abnormal condition existed in the fifth pair of roasts. It may have been that No. 10 was less compact and more loosely bound between the muscle and fat tissue segments than No. 9.

Because of rapid thermal changes in the meat, change in protein structure, and uneven time-exposure to heat during cooking, such data were obviously not so satisfactory for comparing permeability as were ripening shrinkage data, where conditions were maintained as uniform as possible for the same period of time for all cuts.

TENDERNESS

Although the low-calcium roasts were judged more tender in four pairs, three of them showed very narrow and insignificant margins. Comparisons of mechanical shear, collagen content, and palatability grades for tenderness are shown in Table 26.

Collagen was found to be slightly higher in the high-calcium samples in five pairs.

TABLE 26.—COMPARISON OF MECHANICAL SHEAR, COLLAGEN CONTENT, AND PALATABILITY GRADE FOR TENDERNESS ON RIB EYE OF FRESH AND RIPENED RIB ROASTS FROM STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NO.	Mechanical shear on cooked sample		Collagen, fraction total protein		Palatability tenderness		Water-soluble fraction total protein	
	Pounds		Percent		Grade*		Percent	
	Fresh	*Ripe*	*Fresh*	*Ripe*	*Fresh*	*Ripe*	*Fresh*	*Ripe*
1 H	14.2	11.7	5.30	5.52	5.8	6.0	73.8	81.6
2 L	12.9	10.4	3.93	4.73	5.6	5.8	79.7	79.5
3 H	16.3	13.9	4.61	4.58	5.0	5.8	73.0	77.7
4 L	16.0	13.7	4.54	4.23	5.2	5.4	76.3	83.0
5 H	11.6	9.8	4.47	3.66	6.4	6.2	77.5	81.6
6 L	14.4	14.4	4.37	3.90	5.8	5.6	75.2	80.1
8 H	15.5	13.2	4.25	4.53	5.4	5.0	78.1	77.5
7 L	9.7	9.3	4.20	3.45	6.4	6.4	78.5	83.0
10 H	19.2	10.3	4.77	4.94	5.0	5.2	73.1	74.6
9 L	23.2	12.2	4.52	4.24	5.2	5.4	76.5	78.9
12 H	13.3	11.1	4.24	3.23	5.0	6.0	74.5	83.8
11 L	15.4	15.7	5.44	3.60	5.2	5.6	71.2	84.5

* See table 23.

In only one case where a difference in palatability grade of more than 0.2 unit occurred between pair mates did mechanical shear indicate tenderness relationship contrary to palatability grade, in both fresh and ripened samples.

On the same basis of comparison, in only two pairs did more collagen appear in the sample judged more tender by eating. Only one sample (No. 8) was judged more than 0.2 grade unit less tender after ripening. This sample also contained more collagen in the ripened cut than in the fresh one. In fact four ripened samples had more collagen than the corresponding fresh samples. This peculiar condition frequently has been observed and casts some doubt on the uniformity of corresponding right-rib and left-rib cuts from the same beef carcass.

Three samples were judged more than 0.2 grade unit more tender after ripening, and also contained less collagen in the ripened cuts. However, there were too few significant tenderness differences in sample pairs and in fresh and ripened samples to apply a significant estimate of correlation between palatability grade, mechanical shew, and collagen content.

The Mitchell *(34)* water extraction method for collagen and elastin was adapted for the purpose of effecting a gross separation of protein fractions in the rib eye, classified primarily by physical behavior, in order to observe relation of tenderness to other protein fractions than collagen. The fraction passing through a 100-mesh sieve was called "water soluble"; it was made up to volume in a four-liter bottle, and, while the contents were vigorously agitated by a motor stirrer, a 200-ml. sample was taken for Kjeldahl nitrogen.

In some samples a distinct difference was observed in the softness of the residue remaining after the gelatinized collagen had been washed from it. To test the relative dispersability of the residue, it was vigorously agitated five minutes in 400 ml. of boiling water with a motor stirrer and transferred to a 100-mesh sieve. The coarse residue was washed on the sieve with a fine stream of hot water until a combined filtrate of one l. was obtained, which was called the "fine residue,'' and a 100-ml. aliquot was taken for Kjeldahl nitrogen. The coarse residue was treated by trypsin digest as directed by Mitchell. After the elastin had been removed on a 100-mesh sieve, the filtrate was made up to one l. and a 50-ml. aliquot was taken for Kjeldahl nitrogen. This fraction was designated the '"coarse residue."

The ranges of percentage composition of total rib eye protein in the various fractions, both fresh and ripe, are shown in Table 27.

A complementary relation was observed between the water-soluble fraction and collagen, which led to a further observation that collagen was a nearly constant percentage of the water-insoluble fraction. The water-soluble fraction and collagen are shown in Table 26.

The percentage of collagen was found to be very nearly equal to 21 percent of the water-insoluble fraction of the total rib-eye protein. The average deviations in both fresh and ripened samples covering

TABLE 27.—RANGES OF FRACTIONS COMPOSING TOTAL RIB-EYE PROTEIN, IN PERCENT.

FRACTION	Fresh	Ripe
Water soluble	71.20–79.70	74.60–84.50
Collagen	3.90– 5.30	3.20– 5.50
Fine residue	1.20– 3.00	0.80– 1.80
Coarse residue	11.40–17.80	9.00–17.20
Elastin	0.01– 0.04	0.01– 0.04

a period of five years are shown in Table 28. All were steers of approximately the same age (about 18 months) but were of widely different condition.

TABLE 28.—DEVIATION OF COLLAGEN FROM 21 PERCENT OF WATER-INSOLUBLE FRACTION OF TOTAL RIB-EYE PROTEIN.

YEAR	Number of samples		Average of deviation*	
	Fresh	Ripe	Fresh	Ripe
			Percent	*Percent*
1937	8	8	0.38	0.35
1938	14	12	0.34	0.33
1939	12	12	0.57	0.40
1940	11	10	0.31	1.03
1941	11	4	0.49	0.28

* Average deviation of all samples, 0.46 percent.

It was apparent that a satisfactory approximation of the amount of collagen could be made by simply determining the water-soluble fraction and total protein, thereby greatly reducing the time and labor involved. The results should be adequate for many types of routine meat-quality investigations. The group of samples showing greatest deviation were the ripened samples of 1940. These steers were used in the third trial of a phosphorus-feeding experiment described in Part 11, and mention was made of the abnormal ripening performance of those rib cuts.

The relation found between collagen and water-insoluble protein suggested the latter to be a structure of which collagen was a constant fraction serving as the structural binding agent; for as collagen decreased during ripening, more of the complex became water-soluble.

JUICINESS

Palatability grade for quality of juiciness divided preference nearly evenly between the high- and low-calcium roasts. Grade for quantity of juice was higher for the low-calcium roasts in four pairs as shown in Table 29.

TABLE 29.—RELATION OF JUICINESS TO PRESS-FLUID VOLUME AND COOKING LOSSES IN RIB ROASTS FROM STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NUMBER	Palatability grade for juiciness*		Press fluid from 100 g. rib eye in ml.		Cooking losses in percent	
	Quality	Quantity	Raw	Cooked	Drippings	Total
1 H	5.6	5.0	50.0	28.4	1.8	10.0
2 L	4.6	4.4	46.0	22.2	2.5	13.7
3 H	5.2	4.8	49.0	28.6	3.3	14.7
4 L	4.6	5.0	48.3	23.0	3.2	14.2
5 H	5.2	5.6	48.0	21.6	3.5	13.7
6 L	4.8	4.6	50.0	22.0	3.3	16.7
8 H	3.8	5.0	47.7	31.0	1.9	13.9
7 L	4.6	5.4	45.2	31.6	2.0	12.2
10 H	4.4	4.8	54.8	32.0	3.0	17.7
9 L	4.8	5.6	54.2	32.0	1.5	12.0
12 H	4.6	4.8	52.8	28.0	2.6	14.3
11 L	4.6	5.4	51.6	31.0	2.4	13.9

* See Table 23.

However, in the first trial, grade for quantity was higher for the high calcium roasts in three out of four pairs. Therefore, no trend was apparent to distinguish between high- and low-calcium roasts in regard to quality or quantity of juiciness.

No correlation appeared between grade for quantity of juiciness and quantity of press fluid obtained from either raw or cooked rib eye. The small range of variation in quantity of press fluid was further evidence of little distinction in quality between high- and low-calcium roasts. Relation of palatability grade to quantity of press fluid was discussed in Part II.

Higher grade for quality of juiciness coincided with greater amount of drippings in only three pairs, but coincided with lower total cooking losses in four pairs.

A higher concentration of calcium in the press fluid from the high-calcium samples was found in only two pairs, as shown in Table 30. Press-fluid calcium followed the order of total rib-eye calcium in all but the last pair, where the difference was insignificant.

TABLE 30.—PHOSPHORUS AND CALCIUM IN 100 G. RIB EYE AND PER 100 ML. PRESS FLUID FROM RAW RIB EYE. SAMPLES WERE TAKEN FROM 9TH- TO 11TH-RIB CUTS OF STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NUMBER	Total in 100 g. rib eye			Content in 100 g. rib eye		Total in 100 ml. press fluid		
	Phosphorus	Calcium	Ratio P/Ca	Water-soluble inorganic phosphorus		Phosphorus	Calcium	Ratio P/Ca
				Fresh	Ripe			
	Mg.	*Mg.*		*Mg.*	*Mg.*	*Mg.*	*Mg.*	
1 H	202	3.91	51	96	129	187	4.25	44
2 L	201	6.85	39	89	123	193	5.26	37
3 H	202	3.82	53	107	132	199	5.60	36
4 L	205	3.62	56	91	119	199	3.47	57
5 H	206	3.88	53	91	119	198	3.12	63
6 L	206	4.05	51	99	115	192	3.20	60
8 H	207	3.97	52	99	116	192	4.40	44
7 L	203	4.20	48	103	114	188	4.94	38
10 H	201	3.63	55	95	122	202	3.25	62
9 L	204	3.68	55	100	122	206	3.35	61
12 H	206	3.60	57	102	124	203	4.86	42
11 L	203	3.62	56	89	116	208	3.25	64

Five press-fluid calcium concentrations were higher, and seven were lower than the corresponding total rib-eye calcium concentrations but differences were small.

Nine press-fluid phosphorus concentrations were slightly less than total rib-eye phosphorus, but the even balance of phosphorus and calcium in rib eye and its press fluid is illustrated by the fact that six press-fluid P/Ca ratios were higher and six were lower than the corresponding rib-eye ratios, and again differences were small.

Furthermore, no significant variation occurred in the relation of inorganic phosphorus in the rib eye to total phosphorus, total calcium, or calcium in the ration.

Therefore, from these results it seemed evident that no significant difference in juiciness or phosphorus-calcium mineralization of the rib eye was caused by feeding high or low levels of calcium in the ration for steers.

COLOR OF RIB EYE

The gross compositions of rib eyes as shown in Table 31 indicated remarkable uniformity, especially in ether extract and moisture.

The color of five of the samples was bordering on the "shady" class, though probably only one, No. 12, was dark enough to escape classification as light beef. This sample, as shown in Table 32, had the lowest oxidation potential and next to the highest pH. Sample No. 7 had the highest pH and next to the lowest oxidation potential; its color rating (A4+) was also next to the darkest sample, No. 12

(See Table 32). Low oxidation potential and high pH are characteristics of dark beef, which will be discussed in Part IV.

Of the two darkest samples mentioned above, No. 12 was in the high-calcium lot and No. 7 was in the low-calcium lot. However, some significance might be attached to the fact that three of the four brightest samples (color A3) were in the low-calcium lot. The one in the high-calcium lot, No. 5, had next to the lowest amount of muscle hemoglobin, which undoubtedly accounted for a lighter color. Also the dark color reading for low-calcium sample No. 9

TABLE 31.—PERCENTAGE COMPOSITION OF RIB EYE OF 9TH- TO 11TH-RIB CUTS FROM STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NUMBER	Protein	Ether extract	Moisture	Ash	Total
1 H	21.20	0.82	75.75	1.10	98.87
2 L	21.60	0.72	75.40	1.09	98.79
3 H	21.65	1.59	74.50	1.08	98.82
4 L	21.35	1.00	74.80	1.09	99.24
5 H	21.40	1.16	75.10	1.12	98.78
6 L	22.05	1.76	74.60	1.10	99.51
8 H	22.18	0.63	75.30	1.10	99.21
7 L	21.32	0.74	75.80	1.10	98.96
10 H	21.45	1.12	75.80	1.06	99.43
9 L	21.75	0.97	75.65	1.08	99.45
12 H	22.08	1.40	74.50	1.08	99.06
11 L	21.88	1.94	74.40	1.06	99.28

TABLE 32.—COLOR OF RIB EYE AND RELATED FACTORS. SAMPLES WERE TAKEN FROM 9TH- TO 11TH-RIB CUTS OF STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NUMBER	Hemoglobin in blood	Muscle hemoglobin	pH by quinhydrone electrode		Oxidation potential*		Glutathione in 100 g.	Color U.S.D.A. meat scale
			Fresh	Ripe	Fresh	Ripe		
	Percent	*Percent*	*Units*	*Units*	*Volts*	*Volts*	*Mg.*	*Units*†
1 H	11.80	0.404	5.76	6.02	0.151	0.104	11.3	A4+
2 L	15.70	.428	5.87	6.03	.144	.102	12.8	A3
3 H	14.20	.380	5.81	5.98	.132	.134	15.8	A4+
4 L	12.89	.348	5.77	5.97	.142	.109	14.3	A3
5 H	11.70	.352	5.87	6.17	.140	.111	13.7	A3
6 L	13.37	.388	5.86	6.09	.171	.125	15.8	A4
8 H	13.82	.408	5.92	6.14	.149	.092	13.4	A4
7 L	14.89	.428	6.01	6.21	.127	.086	10.1	A4+
10 H	13.93	.372	5.94	6.27	.175	.119	12.8	A4
9 L	12.70	.492	5.95	6.26	.146	.106	11.0	A4+
12 H	13.48	.384	5.98	6.09	.117	.095	11.9	A5
11 L	15.10	0.396	5.88	6.04	0.154	0.129	15.5	A3

* Referred to standard normal hydrogen electrode.

† Munsell—U. S. D. A. standard color units range from A1 (pale pink) through shades of red to A10 (purplish black). Color was graded three hours after cutting.

must have been caused to some extent by its high muscle-hemoglobin content, highest of all.

In view of these circumstances a definite trend toward darker color was evident in the high-calcium samples. In the first limestone-feeding trial, the two darkest samples were in the high-calcium lot, and the brightest one was in the low-calcium lot, indicating a trend similar to that of the second trial.

Since characteristic high pH values in dark beef indicate inhibition of acid-forming enzyme systems in the tissue, and since Mawson *(31)* has shown that addition of glutathione to dialyzed tissue-extracts caused increase in formation of lactic acid from hexosediphosphate, glutathione determinations were made in order to observe if its amount was related to pH and brightness of color.

Although no wide variation occurred in amounts of glutathione, as shown in Table 32, a trend toward low amounts in samples having high pH was evident. The highest pH (in No. 7) was accompanied by the lowest glutathione content, and all pH values of 5.95 or above were accompanied by glutathione amounts under 11.9 mg. per 100 g. rib eye. All but two of the pH values of 5.88 or lower were accompanied by glutathione amounts of 13.7 mg. or more.

Glutathione was determined by a modification of the method described by Huzita and Numata *(24)* adapting it to use with the Bausch and Lomb spectrophotometer. To eight ml. of filtered aqueous extract. of tissue was added two ml. 10-percent HPO_3. The mixture was shaken and filtered. To two ml. of this filtrate in the photometer cup was added in order eight ml. saturated NaCl solution, one ml. freshly prepared two-percent sodium nitroprusside, and one ml. normal ammonia. The mixture was quickly stirred with a thin glass rod, and the optical density at 5300 Angstroms was read within one or two minutes from time of mixing. From standards of pure reduced glutathione used at 26° C. and depth of four cm. the concentration in mg. per 100 ml. filtrate was found to be (optical density –0.12) X 11.95. This value times 1.25 gave concentration in tissue extract.

If optical density exceeded 1.0, fading was too rapid for satisfactory measurement, and less extract was used with sufficient water to make eight ml.

The amounts of glutathione found were lower than the 25 mg. per 100 g. leg muscle of rat reported by Dohan and Woodward *(13)*. However, these samples had been kept in frozen storage 11 months. A sample of fresh beef was found to have 40.5 mg. per 100 g. wet tissue.

Measurements of pH were made with a quinhydrone electrode. Following measurement of oxidation potential, the platinum electrode was removed from the tissue and dipped in powdered quinhydrone, which was then worked into the hole in the tissue by means of the electrode. The tip of the calomel cell was allowed to touch the surface of the slice to complete the circuit. Subsequently parallel measurements with quinhydrone and glass electrodes indicated

TABLE 33.—PERCENTAGE COMPOSITION OF OUTSIDE RIB-FAT TISSUE, AND CONTENT OF PHOSPHORUS AND CALCIUM IN MG. PER 100 G. OUTSIDE RIB-FAT TISSUE. SAMPLES WERE TAKEN FROM 9TH- TO 11TH-RIB CUTS OF STEERS FED ON HIGH AND LOW LEVELS OF CALCIUM.

ANIMAL NUMBER	Percentage by weight				Weights in mgs. per 100 g.		Distribution ratios		
	Protein	Ether extract	Moisture	Ash	Phosphorus	Calcium	P/N	Ca/N	P/Ca
1 H	9.92	74.18	16.40	0.28	42.4	19.47	0.0267	0.0122	2.18
2 L	8.40	77.20	13.88	.26	40.6	18.21	.0303	.0136	2.23
3 H	5.12	83.90	10.70	.19	26.6	9.76	.0328	.0121	2.75
4 L	5.66	84.90	9.87	.20	29.4	10.20	.0327	.0113	2.88
5 H	3.38	88.52	8.98	.15	21.2	8.14	.0392	.0151	2.60
6 L	3.96	87.85	8.79	.14	28.2	16.90	.0448	.0268	1.67
8 H	9.51	73.70	18.67	.29	47.0	15.20	.0309	.0100	3.09
7 L	4.97	84.85	10.30	.17	26.2	5.72	.0327	.0071	4.58
10 H	4.23	85.20	11.53	.17	28.7	12.06	.0422	.0177	2.38
9 L	6.93	78.60	15.42	.24	39.6	13.28	.0357	.0120	2.98
12 H	5.00	85.55	11.34	.17	27.3	9.18	.0341	.0115	2.97
11 L	4.78	84.40	11.48	0.18	27.1	12.44	0.0356	0.0164	2.18

that quinhydrone pH values were 0.3 to 0.4 of a unit higher, occasionally more, than glass electrode values in beef rib eye.

Oxidation potentials were measured as described in Part II.

CHARACTER OF FAT TISSUE

The feeding of two different levels of calcium had no apparent effect on the gross composition or phosphorus-calcium mineralization of outside rib-fat tissue as shown in Table 33. Similar results were obtained in the first trial. These observations are in distinct contrast to those made in the low-phosphorus feeding experiments, in which distinct differences were found in phosphorus-calcium-nitrogen relationships in the outside rib-fat tissue.

(For summary and conclusions, see page 79.)

PART IV. CHARACTERISTICS OF DARK-CUTTING BEEF. SURVEY AND PRELIMINARY INVESTIGATION[6]

INTRODUCTION

In dark-cutting beef, the color of the lean may vary from brownish red (designated as "shady") to purplish black. The appearance of the fat is usually normal. The unattractive and abnormal appearance of the lean naturally causes the consumer to suspect it to be spoiled or from a diseased animal. However, after cooking, it is normal in appearance, and no reason has appeared to cause it to be suspected of being anything but healthful, nutritious, and palatable as bright beef. Its sale usually must be induced by sacrifice in grade and price, which loss ultimately falls upon the processor.

No means has yet been devised for predicting dark color in the meat before slaughter of the animal, and the processor has no means of protection against this loss except as it is offset by more profitable operations. Losses from this one cause amounted to $140,000 in one year for one Chicago packing concern. Chicago packers have estimated their combined yearly losses from this source at approximately one million dollars.

The Ohio Agricultural Experiment Station *(46)* reported the occurrence of 1 percent dark-cutters in a total of 5,993 carcasses in a survey made in Columbus, Ohio, between April 16 and May 27,1939. Maximum price reduction because of color was 3 cent's a pound carcass weight.

The cause of dark beef is not yet known. There have been strong suspicions among packer buyers that certain feeding and management practices, especially grass feeding, caused it. As a result discriminations were sometimes made against cattle sold by certain feeders whose cattle at one time had been known to yield dark-cutters.

On the other hand, among a great variety of assumptions there has been wide belief that delayed bleeding at time of slaughter, and excitement and violent handling of the animals just previous to slaughter were at least contributing causes of dark beef.

The controversial nature of the problem is therefore evident, involving practices of both producers and processors.

SURVEY

Little if any information concerning dark-cutting beef has been published except in confidential reports of a few experiment stations and commercial agencies. Directors of the state agricultural experiment stations, at a meeting in 1925 to consider administration of the Purnell Act appropriation, included as one of the four projects

6. This investigation was conducted in coöperation with and supported by grant in aid from the National Livestock and Meat Board.

in their program of Purnell research one entitled "Factors Involved in the Production and Quality of Meat."

The resulting project was organized as a cooperative effort between the experiment stations and United States Department of Agriculture. The National Livestock and Meat Board has likewise been an active cooperative agency. One of the major phases in the project outline covered an extensive study of color in meat, methods for its measurement, and its dependence on other factors such as feed, age, rate of development, and storage.

Although no specific mention of dark-cutting beef was made in the original project outline, interest in that problem has dominated development of the color phase of the project, and its importance has been emphasized frequently in the discussions at the annual conferences of the cooperative agencies.

In 1932 the authors (19) reported two simple experiments on dark beef which demonstrated that the dark color was not caused by the presence of any abnormal pigment. An aqueous extract of dark beef was found to be normal bright red with normal spectrophotometric characteristics as determined by Shenk, Hall, and King *(48)*. This behavior seemed to indicate that the dark color might be due to incomplete oxygenation of muscle hemoglobin, and that oxygenation was completed during the process of extraction with water.

The sticky, gummy nature of dark beef easily lent itself to the supposition that the tissue was less permeable to oxygen than bright beef; if adequate oxygen could be introduced into it, the color should brighten. Accordingly some finely ground dark beef was placed in a vacuum desiccator (without desiccant) and quickly evacuated with an efficient pump. The vessel was then allowed to fill with oxygen; in a few minutes the meat was perfectly normal bright red. It, therefore, appeared evident that a deficiency of oxygen existed in the dark beef. But it was not clear whether the deficiency was caused by low permeability of the tissue or excessive demand for oxygen by post-mortem metabolic processes.

A number of experiments on delayed bleeding have been reported with somewhat contradictory results. The Illinois Agricultural Experiment Station *(6)* reported one experiment in which spectrophotometric measurements of brightness were invariably lower in animals subjected to 10-minute delayed bleeding; in another experiment, where breeding and feeding varied, the evidence was less conclusive.

The New York (Cornell) Agricultural Experiment Station *(22)* reported finding no darkening due to delayed bleeding, and confirmed their findings the following year *(23)*. A period of five minutes intervened between stunning and bleeding.

The authors *(19) (28)* reported delayed bleeding experiments on two steers in 1932; neither carcass was a dark-cutter, though one was slightly darker than the controls; the other was almost identical in brightness with the controls. The rib eye of the first carcass contained less blood hemoglobin than two of the eight controls, However, it happened to contain more muscle hemoglobin than any of

the controls, which may have accounted for its darker color measurement. Rib eye of the second carcass contained no measurable amount of blood hemoglobin. Bleeding was delayed 10 minutes.

Although the precise effect of delayed bleeding remains uncertain, it seems significant that it caused no truly characteristic dark-cutters as reported in any of the above mentioned experiments.

Little information is available concerning effect of preslaughter excitement on color of beef. The Illinois Agricultural Experiment Station *(7)* conducted an experiment in which 11 calves were run by a man on horseback for 1.25 to 1.5 miles just prior to slaughter. The calves were very hot and exhausted after their run. The rib eyes of the carcasses were found to be unusually bright by visual observation and by spectrophotometric analysis.

Similar results were obtained at the New York (Cornell) Agricultural Experiment Station *(22)*. Animals were tied behind a truck and given a run previous to slaughter, but no dark beef resulted from that treatment.

In 1938 the National Livestock and Meat Board sponsored the organization of a committee for the study of dark-cutting beef. The committee, hereafter referred to as the Meat Board Committee, included chemists and technical staff members from the laboratories of cooperating packing companies and the Institute of American Meat Packers. This committee reported the following case in a survey of the occurrence of dark-cutting beef in 1938 *(37)*.

Rigorous exposure of unfed animals two days before slaughter, with no cause for excitement other than strange environment, resulted in off-color grades in 28 percent (including 11 percent black) of a lot of 53 calves. The animals were exposed to rain and cold without shelter early in December, and had been without feed three days before slaughter. Furthermore, the animals had previously been accustomed to ample provisions and comfortable quarters.

The effect of abrupt change to more rigorous environment requiring higher maintenance energy level appears to be more than mere coincidence. The occurrence of dark beef is rare in summer months, but it rises sharply in the fall following first frosts. Although careful observations have been made on carcasses of animals slaughtered at this station every year for more than 15 years, only once has beef been found dark enough even to be classed as shady *(27)*. In that year the slaughter date (November 7) was one and one-half to two months later than usual. Of the remaining 30 steers of that project which were shipped to Kansas City, three yielded black beef when slaughtered there at a packing plant. The general practices of handling cattle in transit and in the yards previous to slaughter undoubtedly in many cases combine the factors heretofore discussed: chilling, excitement, and rigorous exposure of unfed animals.

Correspondence with packing plant superintendents still reveals persistent ideas that occurrence of dark beef is associated with grass feeding. Nevertheless it is interesting to note that the three black carcasses mentioned above came from the same lot of steers finished

in dry lot on full feed 100 days before slaughter. Included in the same shipment were an equal number of animals full-fed on pasture the same period of time, which yielded no black carcasses. While an isolated example of this sort cannot be accepted as sufficient proof that grass feeding is not a cause of dark beef, it serves as supporting evidence.

Even though grass feeding in itself might not cause dark beef if animals were at the same time getting a grain ration, there is strong possibility that the carbohydrate reserve (glucose and glycogen) in beef muscle tissue might be less in animals fattened on grass alone or with very little grain supplement. This possibility needs investigation, since it will be shown later that carbohydrate reserve is practically non-existent in black beef tissue. Whether a similar condition existed in the animal before slaughter is not known, and an answer to the question is difficult because of the unpredictable occurrence of dark beef.

In the case mentioned above of the two lots of steers, one full-fed in dry lot and the other full-fed on pasture, both should have had ample carbohydrate reserve if that depended on feeding alone. The occurrence of black-cutters in the dry-lot steers suggested other factors than feeding were involved in the cause. The dry-lot steers were provided with sheds. The pasture animals may have been more resistent and better conditioned to withstand rigorous exposure and sudden change of conditions. It is well known that animals maintained under constant environmental conditions, especially mild temperature, have lower resistance to shock from chill and exposure than animals regularly subjected to wide temperature fluctuations.

There was, however, a very significant difference in these lots in regard to exercise. The dry-lot group showed the usual symptoms of heavy feeding combined with little exercise; the pasture group did not. This difference may have been a contributing factor in developing the condition leading to dark colored muscle tissue.

Most 4-H Club calves get only a limited amount of exercise; occurrence of dark-cutters has been found exceptionally high in this group of animals exhibited at the International Livestock Exposition in Chicago *(39)*. Furthermore, questionnaires indicated that on the average less exercise was given the dark-cutters in this group than was obtained by the normal animals, and that the dark-cutters were given 37 percent more roughage in their ration.

A tentative conclusion derived from the foregoing observations indicates the possibility that two conditions may be contributing factors in the occurrence of dark beef: inadequate carbohydrate reserve, and inadequate conditioning of the animals to cultivate resistance against shock from drastic changes in environmental conditions. There is obvious need for investigation of the effect of shock from exposure on carbohydrate reserve in beef muscle tissue, both in grain-fed and grass-fed animals.

The physico-chemical characteristics typical of dark beef first to be announced were low oxidation potential, high pH, high oxygen uptake, and low reducing sugar content.

Relation of low oxidation potential to dark color in beef was observed at this station *(27)* in 1933 and confirmed by the Meat Board Committee *(39)* in 1939.

Relation of high pH to dark beef was observed independently and announced almost simultaneously in 1939 by this station *(29)*, by the Meat Board Committee *(37)*, and by Winkler *(50)*. Winkler apparently made no observations on natural dark beef; but by injecting ammonia into the tissue he was able to demonstrate that a rise in pH was accompanied by a darkening of color.

Difference in relative rates of oxygen uptake in bright and dark beef was first reported by the Meat Board Committee *(37)* in 1939. When adjusted to the normal pH of living tissue (pH 7.4), five black beef samples averaged 30 percent less oxygen consumption than the average of three light samples. However, later in the same year the same investigators *(38)* found in working with only one sample each of dark and bright beef that the relative rate of oxygen uptake depended on the pH, as reported by Canzanelli and others *(8)*. At pH 7.4 the rate for the dark sample was 35 percent less than the rate for the light one. At pH 6.4 (characteristic of black beef) the rate for the dark sample was 75 percent greater than the rate for the light one. At pH 5.4 (characteristic of light beef) the rate for dark beef was more than five times as great as that of light beef. However at the same time the rates for both samples decreased as pH decreased, and decreased more rapidly in the light sample. The dark sample used was induced by insulin injection as described later.

A short time later the same investigators *(39)* found the average oxygen uptake of five natural black samples with average pH 6.6 to be more than five times as great as the average uptake of two light samples with pH 5.6. These measurements were made at the individual native pH of the tissues.

While these results still leave unanswered the question whether oxygen uptake of natural dark samples behaved toward pH changes in a manner, similar to the induced sample mentioned above, and the question as to how much the difference in uptake was due to pH difference and how much due to difference in metabolic demand for oxygen, nevertheless, the results indicate the actual practical relative performance of light and dark beef. And they probably indicate the mechanism by which the dark color is developed. **The demand for oxygen in dark beef is evidently greater than can be supplied by normal transfusion through the tissue, resulting in robbing oxyhemoglobin of its oxygen.**

Along with their first observations on oxygen uptake the Meat Board Committee *(37)* also made the significant observation that black beef contained approximately one-sixth as much reducing sugar (glucose) as light beef. This observation was later substantiated by the authors as subsequently shown. These results naturally infer that rapid oxygen consumption and glucose disappearance were interdependent phenomena. However, proof was lacking that normal amounts of glucose were originally present in dark beef animals before and just after slaughter.

Upon that proof depends another important question, whether restricted acid formation in dark beef depends on abnormally low glucose and glycogen content or a shift in the mechanism of conversion to the formation of less acid products by derangement of some enzyme system.

The next step by the Meat Board Committee *(38)* was to deplete the animals' tissues of glucose by injections of insulin. Animals were slaughtered when sufficient insulin had been administered to produce coma. In four animals thus treated, dark colored rib eyes, chucks, and rounds were found. The rib eyes averaged pH 6.0, had half the normal amount of glucose, consumed about twice the normal amount of oxygen, and the color scale values were in the narrow range of A7 to A8, classified as dark beef. This appears to have been the first successful attempt to produce dark beef experimentally, and it marked a significant advance in the study of the problem.

Some questions naturally arise concerning relation between insulin and dark beef:

1. Was glucose depletion in the tissue alone the cause of dark beef through removal of acid forming material?
2. Was the mode of glucose conversion altered so that, less acid products developed?
3. Was abnormally high insulin activity characteristic of animals from which naturally occurring dark beef was obtained?

The first two questions are somewhat interdependent, and it seems significant that there was still half the normal amount of glucose present and twice the normal oxygen uptake. There seemed to be ample opportunity for formation of acids by normal processes. But if the processes were altered so that the acids themselves were not produced as rapidly or were in turn oxidized more rapidly, pH of the tissue would naturally be higher. This question deserves further investigation. Unfortunately glycogen was ignored in these investigations, since its role in post-mortem lactic acid formation was not then understood.

The third question was approached by the Meat Board Committee *(39)* in a later experiment. Pancreas from a large number of animals were prepared for histological examination. When the carcasses were graded, the pancreas corresponding to seven black and seven bright carcasses were examined as to number and size of Islets of Langerhans, beta cells and granules, and alpha cells and granules. Variations were detected in the microscopic preparations, but no particular condition could be related to occurrence of light or dark color in the muscle tissue. Still the investigators felt their observations were not conclusive enough to preclude a possibility that differences may have existed between bright and dark beef in regard to insulin activity.

Little conclusive information has appeared concerning any difference in the nature of acid substances in light and dark beef. The Meat Board Committee *(40)*, using three samples each from light, medium, and black carcasses, found no distinct difference in the

amount of lactic acid. However, much less pyruvic acid was found in black beef than in the two other color classes. If this observation can be confirmed with significant numbers of samples, it constitutes striking evidence of the nature of the difference in glucose conversion in bright and dark beef.

The effects of feeding increased amounts of protein, carbohydrate, and fat were investigated in relation to color of beef in an experiment sponsored by the National Livestock and Meat Board *(41)* at the Kansas Agricultural Experiment Station. Steers in lot 1 (controls) received a typical corn-belt ration. Lot 2 received cottonseed meal, lot 3 received Cerelose (corn sugar), and lot 4 received cottonseed oil, each substituted for part of the corn in the basal ration so that the four rations were identical in caloric value.

Two steers from each lot were slaughtered at the Kansas station in mid-March, and the remaining eight steers from each lot were shipped to Chicago where they were kept in open pens exposed to cold weather (28° to 5° F) and without feed until time of slaughter. Average meat scale color readings of the cattle slaughtered in Chicago were as follows: Lot 1, 2.87; lot 2, 4.12; lot 3, 4.00; and lot 4, 4.75. Two members of lot 4 registered the darkest rib-eye color reading, 6, of all 32 carcasses.

While this color was classed as shady, no typical dark or black beef developed. In lot 4 the highest average pH was found, also the lowest average oxidation potential. The lowest individual, but not lowest average reducing sugar, also occurred in lot 4. These factors correlate with the color readings to substantiate a trend toward darker color in the lot receiving cottonseed oil. The oil was fed at the rate of one-third pound daily to each animal in lot 4. The highest individual and highest average reducing sugar were found in lot 3, but not a corresponding lightness in color.

The results of the above experiment illustrate the adage of biochemistry that "fats burn in the flame of carbohydrate," probably thus accounting for lower amounts of glucose in the muscle tissue of lot 4. This condition again was associated with darker color, although not to the extent found in the insulin experiment previously mentioned. But again it is not clear whether lower acid development in the tissue was caused by deficiency of carbohydrate or formation of less acid oxidation products under the influence of additional fat oxidation.

EXPERIMENTAL PROCEDURE

From the foregoing discussions it seemed that the abnormally high pH of dark beef was significantly related to its abnormal carbohydrate conversion and abnormal physical behaviour. It seemed probable that any derangement of normal acid development in beef muscle tissue resulting in higher pH might cause darker color. It therefore seemed important to find out whether changes in pH of dark beef and bright beef would be accompanied by similar physical changes in both, and whether both had the same isoelectric point.

The extremely lyophylic nature of dark beef and the high degree of colloidal dispersion in aqueous extracts from it have been reported from this station *(19), (20)*. In the present investigation water imbibition measurements were made on light and dark beef from pH 4 to pH 7.6. The isoelectric point was determined by the region of minimum imbibition.

Pre-slaughter and post-mortem progress of glucose changes in blood and muscle tissue was observed during 10 days before and five days after slaughter of a steer-receiving only water.

Post-mortem changes in pH, glucose, glycogen, hydrolyzable sugar, inorganic phosphorus, lactic acid, and pyruvic acid were followed over a period of eight days in a steer full-fed until time of slaughter.

Rates of conversion of glucose and glycogen were determined by incubation of tissue preparations of bright and dark beef adjusted to the same pH.

Buffer strength titrations were made on samples of light and dark beef with sodium hydroxide, hydrochloric acid, lactic acid and pyruvic acid.

Generation of lactic acid in dark beef was measured during incubation with added glucose and glycogen.

WATER IMBIBITION AND ISOELECTRIC POINT

By altering the pH of a series of portions of the same beef rib eye it was found that the amount of imbibed water could be varied from 110 percent at pH 7.60 to 27 percent at pH 5.00, where it appeared to be at a minimum as shown in Table 34. With further decrease in pH, amount of imbibed water increased.

A sample of black beef supplied by Wilson and Company in Kansas City exhibited a minimum at practically the same pH as the bright sample. This point is clearly shown in Figure 1.

Filtrates from both samples in the region of pH 5 were almost completely free from turbidity. But turbidity increased on either side of this point, in both sample series. Therefore, it seemed justifiable to conclude that the isoelectric point of black beef muscle protein was the same as that of bright beef, and was near pH 5.07. The physical properties of both samples were quite similar at the same pH.

The concentration of inorganic phosphorus found in the aqueous extract, as shown in column 5 of Table 34, exhibited divergent tendencies in black and bright samples in relation to pH changes on the alkaline side of the isoelectric point. Inorganic phosphorus remained practically constant up to pH 6 in the bright samples, and then it sharply dropped. In contrast there was a steady increase in inorganic phosphorus concentration in the black beef extracts as the pH increased. The final values in the two series were not reliable because of the small amount of extract obtained for analysis.

Although insufficient supporting evidence was available to establish the significance of these results, it seemed evident that the degree of phosphate retention (probably by adsorption) was different

TABLE 34.—EFFECT OF pH ALTERATION ON WATER IMBIBITION AND INORGANIC PHOSPHORUS EXTRACTION IN BRIGHT AND BLACK BEEF RIB EYE.

SAMPLE NUMBER	Acid or alkali added	pH	Imbibed water	Inorganic phosphorus	
				In four ml. extract	In extract from 100 g. rib eye‖
	Ml.	*Units*	*Percent*	*Mg.*	*Mg.*
1 A*	3.0 acid‡	4.20	60	3.88	56
B†	5.0 acid	4.00	52	5.95	108
2 A	2.0 acid	4.60	38	4.10	80
B	4.0 acid	4.32	28	6.15	143
3 A	1.0 acid	5.00	27	4.27	86
B	3.0 acid	4.69	22	6.25	144
4 A	0.5 acid	5.30	28	4.39	84
B	1.5 acid	5.44	26	7.11	145
5 A	0.0	5.70	33	4.39	76
B	1.0 acid	5.90	40	7.49	121
6 A	1.0 alk.§	6.40	52	2.87	39
B	0.5 acid	6.34	64	7.95	75
7 A	2.0 alk.	6.90	96	2.91	14
B	0.0	6.77	76	8.15	49
8 A	3.0 alk.	7.60	110	4.08	4
B	1.0 alk.	7.30	102	6.89	5

* A, bright.
† B, black.
‡ Acid, 1 *N* H_2SO_4.
§ Alkali, 0.75 *N* NaOH.
‖ Normal content of original, unaltered rib-eye samples:
Total phosphorus: bright, 201; black, 215 mg./100 g. rib eye.
Inorg. phosphorus: bright, 89; black, 165 mg./100 g. rib eye.

in black and bright beef, not only quantitatively, but also qualitatively. It is interesting to note that the concentration of inorganic phosphorus in bright beef extract passed through a maximum near pH 5.5, which is the normal pH of bright beef; also in the black beef extract the concentration passed through a maximum at the native pH of the tissue, 6.77, although the value of the one point beyond it was doubtful and thereby caused the maximum to be in doubt. However, it seemed that addition of either acid or base at the native pH caused closer retention of inorganic phosphates in the muscle tissue, reflected by lower concentration in the extract.

The procedure was as follows: Ground rib eye in 20 g. portions with 20 ml. water was dispersed three minutes with a small high-speed motor stirrer in square four-ounce bottles for effective turbulence. The acid or alkali was added dropwise during the stirring. After standing six hours in a near-freezing cooler, the samples were dispersed again three minutes. The following morning the samples were dispersed three minutes and transferred to centrifuge tubes. During the transfer, pH was measured with a glass electrode potentiometer. Samples were centrifuged 10 minutes at 2,500 r.p.m., all eight samples of one series at once. Liquid was drained off immedi-

ately and measured. This volume was subtracted from total volume of liquid added (including acid or alkali) to indicate volume imbibed by the tissue.

Inorganic phosphorus was determined in the extract by the Fiske and Subbarow *(14)* method for blood.

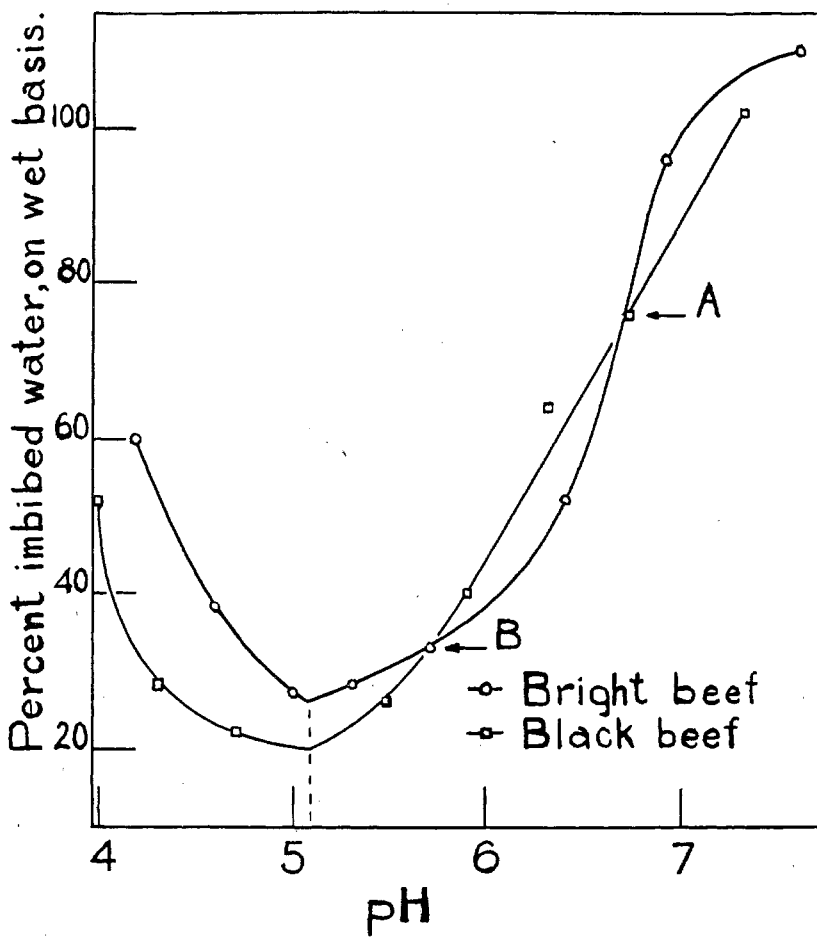

FIG. 1.—Effect of pH alteration on water imbibition by bright and black beef muscle. A. Unaltered pH of black beef. B. Unaltered pH of bright beef.

GLUCOSE CHANGES DURING STARVATION AND AFTER SLAUGHTER

A steer which had been on full feed was taken off feed and given only water for 10 days. Blood samples were taken, and cores of muscle tissue were taken from the right hind quarter by operative technic by staff of the veterinary hospital. Schedule of samples and corresponding amounts of glucose found are shown in Table 35. A decisive drop in glucose occurred in both blood and muscle tissue in the first three days off feed; here blood glucose was at, a minimum, but muscle glucose reached a minimum on the sixth day.

TABLE 35.—CHANGES IN REDUCING SUGAR (AS GLUCOSE) IN BLOOD AND MUSCLE TISSUE (ROUND) BEFORE AND AFTER SLAUGHTER OF STEER RECEIVING ONLY WATER.

Elapsed time after last feed	Glucose in 100 ml. serum	Glucose in 100 g. muscle tissue (round)
Days	*Milligrams*	*Milligrams*
0	95	.....
1	82	108
3	50	33
	70*	.....
6	55	26
8	53	66
	69*	.....
10	82	61
	72*	.....
	Slaughtered	
10	34†	44
11	.....	118
13	.....	130‡
		108
14	.....	170‡
		95
15	.....	63§

* Bled after removal of tissue sample. Other blood samples were taken before removal of tissue.

† Glucose in urine removed from bladder after slaughter.

‡ Sample was taken from left quarter. Other samples were taken from right quarter, which later developed some spoilage.

§ Rib eye sample.

Thereafter both rose, and at the time of slaughter blood glucose was normal. After slaughter, muscle glucose in the left quarter rose in six days to a value nearly normal for bright beef, and the tissue had in fact no indication of dark color. Rib-eye glucose, however, at the end of the period was considerably below normal, but pH and oxidation potential were quite normal. The rib eye likewise was normal bright color.

The amount of glucose found in urine removed from the bladder after slaughter was of course low, but occasionally lower values, usually accompanied by high urine volume, have been found in this laboratory for steers on full feed.

Two significant points can be made from these results. First, low glucose concentration in the rib-eye tissue was not accompanied by

dark color; this fact indicated that the occurrence of low glucose values in dark beef is probably secondary to other abnormal conditions. Second, glucose reached near-minimum concentration in blood and muscle tissue in three days off feed. This observation recalls the case mentioned earlier in this paper wherein 28 percent of a lot of 53 calves were off color when they had been off feed three days and exposed to cold rain previous ,to saughter. Apparently they had been killed just before the mechanism got under way to replenish the glucose from the glycogen reserve in the liver or muscle tissue.

Glucose was determined by a modification of the Folin-Wu *(15)* method using 10-percent cadmium sulfate in N/4 sulfuric acid as the deproteinizing agent. Since uric acid, glutathione, and other substances that reduce alkaline copper tartrate, except glucose and its phosphates, are removed *(18)*, the method is applicable to tissue, blood, and urine.

Enough water was added to sample of aqueous muscle extract (8 ml.), blood (1 ml.), or urine (5 ml.) to make total volume of eight ml. in 50-ml. Erlenmeyer flasks. One ml. cadmium sulfate reagent was added, followed by one ml. 0.75 normal NaOH slowly and with gentle rotation of flask. The mixture was shaken vigorously, and after standing a few minutes it was filtered through a dry quantitative paper. The remainder of the procedure was followed as in the Folin-Wu method, except the tubes were heated 15 minutes in the boiling water bath.

POST-MORTEM CHANGES IN CARBOHYDRATE METABOLITES

Samples of rib eye were taken from a full-fed steer as soon as possible (two hours) after death, and at intervals over a period of eight days. Results of the analyses are shown in Table 36.

TABLE 36.—POST-MORTEM CHANGES IMMEDIATELY FOLLOWING SLAUGHTER OF FULL-FED STEER. CONTENT OF 100 G. RIB EYE.

SAMPLE NUMBER	Hours after slaughter	pH	Weights in milligrams					
			Inorganic phosphorus	Glucose	Glycogen	Hydrolyzable sugar as glucose	Lactic acid	Pyruvic acid
1	2.0	6.80	106	30	706	404	7	69
2	7.5	6.08	85	40	571	500	12	91
3	12.7	5.60	68	62	221	297	18	115
4	26.7	5.55	59	63	205	296	11	93
5	55.5	5.70	77	86	272	261	9	80
6	122.0	5.62	84	80	246	310	12	58
7	194.0	5.62	80	112	244	143	6	58

The pH had already dropped at least one-half unit below the normal living-tissue value when the first sample was taken, and it continued to drop rapidly during the first 12 hours. Thereafter, pH remained practically constant. Most of the other factors reached a minimum, a maximum, or a level in the first 12 hours.

Glucose concentrations were similar to concentrations in the tissue of the starved steer (Table 35) and increased in a similar manner following slaughter. Glycogen made a precipitous drop in the first 12 hours. Lactic and pyruvic acids reached a maximum in the same period, but lactic acid concentrations were undoubtedly much too low and were given only to indicate trends. The method used was for fresh tissues containing far less amounts of these acids *(16)*. Later trials indicated 700 to 900 mg. lactic acid in 100 g. beef muscle tissue at least five days after slaughter. The method is discussed later.

Hydrolyzable sugar (as glucose) did not show a satisfactory correlation with glycogen. In only three of the samples was there more hydrolyzable sugar than glycogen. In those three samples glycogen (as equivalent glucose) averaged 83 percent of the hydrolyzable sugar. Apparently incomplete hydrolysis was attained in the other samples. Hydrolysis was attempted by heating the ground tissue with 2/3 N sulfuric acid. The tissue protein may have buffered the acid to an ineffectively low hydrogen ion concentration. It probably would have been better to prepare a boiling water extract of the tissue for hydrolysis than to follow the above procedure used by the Meat Board Committee.

Glycogen was determined by a modification of Pfluger's *(42)* method. Rib-eye samples of 5 g. were dissolved in 20 ml. 30-percent, KOH over a steam bath with occasional stirring 20 to 30 minutes until well dispersed, though not necessarily clear. After samples were cooled, 15 ml. water and 40 ml. 95-percent ethyl alcohol were added with stirring. After standing over night at room temperature, the glycogen was centrifuged in 50-ml. tubes and washed three times with 25-ml. portions of 66-percent ethyl alcohol. The residue was extracted by centrifugation three times with 10-ml. portions of boiling water.

The extracts were collected in a one-inch test tube, and two ml. of 10 N sulfuric acid were added. The tubes were provided with reflux funnels, and were set deep enough in a steam cone so that the entire liquid portion was exposed to live steam when the tops of the tubes and cone were blanketed with a towel. Occasionally incomplete hydrolysis occurred when these precautions to ensure near-boiling temperature were not followed.

After three hours' hydrolysis the solutions were neutralized to litmus with 30-percent NaOH (about 3 ml.) and made up to 50-ml. volume. Aliquots of two ml. were taken for Foln-Wu glucose determination as described previously. Glucose was multiplied by 0.90 to obtain glycogen.

It was found that use of HCl for hydrolysis, as indicated by Babers *(2)*, caused the phosphomolybdate blue color to fade rapidly, 23 percent, in 70 minutes after addition of the phosphomolybdic acid. Similar effect was observed upon adding a corresponding amount of NaCl to a pure glucose solution before analysis.

The use of KOH for neutralization caused a 3 percent stronger phosphomolybdate blue color in 20 minutes after addition of phosphomolybdic acid. Hydrolysates prepared as described above held a constant color relation toward a standard glucose solution more than 90 minutes.

CONVERSION OF GLYCOGEN IN BRIGHT AND DARK BEEF

Losses of glycogen during incubation of five pairs of bright and dark beef samples were consistently greater in the bright samples when glycogen was added to bring all inital concentrations up to 600 mg. per 100 g. tissue. It was uncertain, however, whether added and native glycogen were converted at the same rate. Since the dark samples contained no glycogen, and the bright ones contained 130 to 230 mg. per 100 g., a difference in conversion rates in native and added glycogen easily could obscure any difference in conversion rates in bright and dark beef.

Furthermore, after doing these experiments, it was found that something happened to glycogen almost as soon as it was dispersed in water with dark muscle tissue, so that only 85 to 90 percent of it was recoverable by immediate analysis. It made little difference whether dispersion was done by gentle stirring with a glass rod or by vigorous agitation with a high-speed motor stirrer. Quantity of glycogen added to the meat was based on analysis of the stock glycogen carried through the complete routine as in meat analysis.

However a differential method was devised for estimating the rate of conversion of added glycogen alone during incubation of dispersed aqueous preparations of muscle tissue as shown in Table 38. Characteristics of the two beef samples used are shown in Table 37.

TABLE 37.—CHARACTERISTICS OF BEEF RIB-EYE SAMPLES OBTAINED FROM WILSON AND COMPANY, CHICAGO.

Sample No.	W15 A10	W16 A2
Color	(black)	(bright)
U. S. carcass grade	choice	choice
Carcass wt., lbs	720	741
pH of ground tissue	6.60	5.53
pH aqueous dispersion	6.59	5.51
Glycogen, mg./100 g.	2	124
Glucose, mg./100 g.	32	228
Inorganic phosphorus mg./100 g.	103	116

TABLE 38.—CONVERSION OF ADDED GLYCOGEN BY BRIGHT AND BLACK BEEF RIB EYE BY DIFFERENTIAL METHOD. DUPLICATE PREPARATIONS WERE ANALYZED IMMEDIATELY WITHOUT INCUBATION. BRIGHT SAMPLES WERE ADJUSTED TO pH OF BLACK SAMPLES, 6.59. INCUBATION PERIOD ONE HOUR AT 38° C.

SAMPLE NUMBER	Prep. No.	Glycogen in 100 g. wet rib-eye tissue					
		Native	Added	Immed. anal.	After incub.	Incub. loss	Total loss
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*	*Percent*
W15.....	1	2	103	.....	75	.....	29
(Black)	2	2	206	188	159	15	24
W16.....	3	124	0	.....	88	.....	29
(Bright)	4	124	103	230	185	20	19

In order to differentiate between incubation losses for *added* glycogen and total glycogen as shown above in Table 38, losses in *added* glycogen were assumed to be represented by the increment loss between preparations No. 1 and No. 2 for the black sample, and by the increment loss between preparations No. 3 and No. 4 for the bright sample. These increment losses were calculated in the following manner from the difference in glycogen concentrations in the two preparations following incubation and the difference in amount of glycogen originally added to the two preparations:

W15 (black). Increment loss between preparations No. 1 and No. 2: Increment added (103)—increment after incubation (159-75) = increment loss (19) = 18.5 percent loss.

W16 (bright). Increment loss between preparations No. 3 and No. 4: Increment added (103)—increment after incubation (185-88) = increment loss (6) = 5.8 percent loss.

Samples of five g. ground tissue with 20 ml. water, added glycogen, and an amount of 0.75 N NaOH to adjust pH of bright sample to pH of dark sample were dispersed four minutes with a high-speed stirrer in square four-ounce bottles. The bottles were filled with oxygen, capped, and placed in a shaker mounted in a Warburg apparatus operating at 120 r.p.m. and four cm. amplitude. Incubation proceeded one hour at 38° C., and immediately afterward seven g. KOH pellets were added to each bottle and quickly stirred into solution. The tissue was dissolved, and glycogen analyses proceeded as described above.

Each preparation in Table 38 represented the average of duplicates. Duplicate pairs of preparations 2 and 4 were analyzed immediately after being made up, disclosing a 9-percent loss in the dark sample, but none in the bright one.

Incubation loss was designated as the difference between amounts found by immediate analysis and after incubation, expressed as percentage of the amount found by immediate analysis. Total loss represented loss to the sum of added glycogen plus native glycogen following incubation.

The method of differentiating incubation losses in *added* glycogen is shown in Table 38. Since added glycogen was converted more rapidly in the black sample, and since a higher incubation loss of

all glycogen occurred in the bright sample, it became apparent that native glycogen was converted more rapidly than added glycogen in the bright sample.

Unfortunately there seems to be no way to compare native glycogen conversion rates in black and bright beef, for the reason there is practically no native glycogen in black beef. However, it is interesting to note that relative conversion rates so compensated each other that percent total losses were the same in preparations 1 and 3.

GLUCOSE CONVERSION IN BRIGHT AND DARK BEEF

An experiment pattern similar to that used in measuring glycogen conversion was used to measure relative rates of glucose conversion in bright and black beef muscle tissue. At the same time an attempt was made to measure changes in content of inorganic phosphorus, lactic acid and pyruvic acid.

Preparations were made with 10 g. ground tissue dispersed in 30 ml. water with added glucose and amount of 0.75 N NaOH to adjust pH of bright sample to pH of dark sample. Immediately after incubation, pH was measured with a glass electrode potentiometer. The material was then transferred to 50-ml. centrifuge tubes, immersed in boiling water five minutes, and frequently stirred. After centrifuging five minutes at 2,500 r. p. m., the extract was poured through ashless filters into 100-ml. volumetric flasks. The tissue was washed twice by centrifugation with 20 ml. boiling water.

TABLE 39.—CONVERSION OF ADDED GLUCOSE BY BRIGHT AND BLACK BEEF RIB EYE BY DIFFERENTIAL METHOD. DUPLICATE PREPARATIONS WERE ANALYZED IMMEDIATELY WITHOUT INCUBATION. BRIGHT SAMPLES WERE ADJUSTED TO pH OF BLACK SAMPLES, 6.59. INCUBATION PERIOD SIX HOURS AT 38° C. NATIVE GLYCOGEN EQUIVALENT IN GLUCOSE: 138 MG./100 G. IN BRIGHT SAMPLE, AND 2.2 MG./100 G. IN BLACK SAMPLE.

Sample Number	Prep. No.	Final pH	Glucose in 100 g. wet rib eye				
			Added	Immed. anal.	After incub.	Incub. loss	Total loss
		Units	*Mg.*	*Mg.*	*Mg.*	*Percent*	*Percent*
W15 (Black)	1	6.59	0	32	22	35	35
	2	6.36	200	183	20	89	35
	3*	6.31	400	333	142	58	65
W16 (Bright)	4	6.51	0	228	222	39	39
	5	6.51	200	432	375	38	34
	6	6.50	400	585	509	30	34

Incubation losses in added glucose

Added glucose	W15 (black)		W16 (bright)	
	Milligrams	*Percent*	*Milligrams*	*Percent*
200	202	101	47	23.5
400*	280*	70*	113	28.2

* Sample caluculation (Prep. No. 3): Increment added (400) — increment after incubation (142-22) = increment loss (280) = 70 percent loss.

TABLE 40.—INORGANIC PHOSPHORUS CHANGES DURING INCUBATION OF PREPARATIONS INDICATED IN TABLE 39.

SAMPLE NUMBER	Prep. No.	Inorganic phosphorus. Milligrams per 100 g. rib eye		
		Immediate analysis	After incubation	Incubation change
W15 (Black)	1	103	157	+54
	2	104	149	+45
	3	103	152	+49
W16 (Bright)	4	118	120	+ 2
	5	117	119	+ 2
	6	120	118	— 2

TABLE 41.—LACTIC ACID CHANGES DURING INCUBATION OF PREPARATIONS INDICATED IN TABLE 39.

SAMPLE NUMBER	Prep. No.	Lactic acid. Milligrams per 100 g. rib eye		
		Immediate analysis	After incubation	Incubation change
W15 (Black)	1	8	6	— 2
	2	5	5	0
	3	5	6	+ 1
W16 (Bright)	4	30	10	—20
	5	30	7	—23
	6	19	12	— 7

TABLE 42.—PYRUVIC ACID CHANGES DURING INCUBATION OF PREPARATIONS INDICATED IN TABLE 39.

SAMPLE NUMBER	Prep. No.	Pyruvic acid. Milligrams per 100 g. rib eye		
		Immediate analysis	After incubation	Incubation change
W15 (Black)	1	43	21	—22
	2	42	23	—19
	3	41	36	— 5
W16 (Bright)	4	31	52	+21
	5	33	60	+27
	6	41	41	0

The extract was made up to 100-ml. volume, and two ml. was taken for inorganc phosphorus determination, eight ml. for glucose determination, and the remaining 90 ml. was taken for lactic and pyruvic acid determinations. Determinations were made as described above. Results are shown in Tables 39, 40, 41, and 42.

Incubation losses in Table 39 represented algebraic differences between analyses of duplicate preparations before and after incubation, plus glucose equivalent of native glycogen. The previous gly-

cogen conversion experiments indicated that glycogen was converted much more rapidly than glucose. Percentage incubation losses were nearly the same in preparations 1 and 4, where no glucose was added, although there was wide difference in initial content of native glucose and glycogen in the black and bright samples.

However *added* glucose was converted much more rapidly in black beef than in bright beef, possibly indicating a difference in rate of phosphorylation in the two samples of beef.

Further evidence of dissimilarity in phosphorus relations in the two samples was shown in the large amount of inorganic phosphorus (phosphate) liberated during incubation of the black samples, while practically no change occurred in amount of inorganic phosphorus in the bright sample. These results might seem to indicate greater phosphatase activity in the black sample. Phosphatase activity naturally would be assumed to interfere with phosphorylation of sugar. Further investigation of phosphatase activity is planned.

As previously mentioned, results for lactic acid and pyruvic acid were uncertain, but they are shown for what they might indicate in trends during incubation.

A distinct difference was noted in the conversion trends of both acids in bright and black beef. Lactic acid remained practically unchanged in the black sample, but decreased in the bright sample nearly to the same level as in the black sample.

On the contrary, pyruvic acid indicated not only a pronounced decrease in the black sample, but an increase in the bright one. These trends are consistent, with the Meat Board Committee's *(40)* observations previously mentioned, in which less pyruvic acid was found in black beef than in bright beef, but little difference was found in lactic acid in the two types of beef.

BUFFER STRENGTH OF BRIGHT AND DARK BEEF

Buffer strength titrations were made on preparations similar to those used for incubation; the purpose was to find out how much lactic or pyruvic acid was necessary to cause a difference in pH corresponding to the pH difference in black and bright beef. It was found that pH change was nearly linear with amounts of lactic and pyruvic acid added between pH 5.50 and 6.50. In that range buffer strength was indicated by the milliequivalents of acid or base necessary to cause a change of one pH unit.

The results shown in Table 43 indicated no significant difference in buffer strength of dark and bright beef toward strong acid and base. The effectiveness of the buffer action toward the strong acid and base can be illustrated by the fact that acid and basic ion concentrations were reduced approximately to 1/15,000 of original after dispersion with the muscle tissue. It was therefore not surprising that similar amounts of lactic and pyruvic acids were required to produce the same pH change caused by HCl and NaOH.

The amounts of lactic and pyruvic acids necessary to cause one pH unit change corresponded approximately to 450 mg. per 100 g.

TABLE 43.—BUFFER STRENGTH OF BRIGHT AND DARK BEEF RIB EYE TOWARD STRONG ACID AND STRONG BASE, AND TOWARD LACTIC AND PYRUVIC ACIDS. ACID OR BASE REQUIRED TO CAUSE pH CHANGE OF ONE UNIT IN 100 G. RIB EYE.

SAMPLE NUMBER	Color	pH of tissue	Acid or base added	Milliequivalents added
A2	dark	6.20	HCl	4.38
A3	bright	5.50	NaOH	4.94
W15	black*	6.60	HCl	4.89
W16	bright†	5.53	NaOH	4.60
W3	black	6.82	lactic	6.58
W10	dark	6.20	lactic	4.80
W10	dark	6.20	lactic	4.84
W6	black‡	6.52	pyruvic	5.55
W8	black	6.48	pyruvic	5.13
W10	dark	6.20	pyruvic	4.12

* A10 U. S. D. A. meat color scale: A1, light pink, through shades of light and dark red to A10, purplish black.

† A2.

‡ A9.

tissue. If formation of lactic acid were the principal cause of post-mortem lowering of pH in normal beef tissue, nearly 900 mg. per 100 g. tissue would be required. The experiment on post-mortem changes (Table 36) indicated sufficient glycogen probably existed in the muscle tissue at the time of death to produce such an amount of lactic acid.

However, when attempts were made to recover lactic and pyruvic acids which had been added in such large amounts, sometimes even less was found than the original native content. Formation of a precipitate was observed in the distillation flasks. It was assumed to be calcium phosphate, and the acidity of the reaction mixture during oxidation to acetaldehyde had probably been lowered by the relatively large amount of calcium lactate.

The method used by the Meat Board Committee for lactic and pyruvic acid determinations was followed in attempt to duplicate the committee's observations that more pyruvic acid occurred in bright beef than in black beef. The method was essentially the one described by Friedemann and Graeser *(16)*. The boiling water extract from 50 g. wet muscle tissue was carried through the routine for lactic acid in urine as described in Practical Physiological Chemistry by Hawk and Bergeim *(21)*. An aliquot corresponding to five g. tissue was oxidized to the aldehyde.

Following the above observations on incomplete recovery of added lactic acid, one ml. of 85-percent phosphoric acid was added to each distillation flask; the yield of lactic acid was greatly increased, but still it was much too low.

An aqueous extract prepared as usual was deproteinized with tungstic acid. Although a 100-ml. aliquot would have been used in the above method, smaller aliquots were tried. Aliquots of 20 ml. yielded 332 mg. lactic acid per 100 g. tissue; aliquots of four ml. yielded 916 mg. per 100 g. tissue, which seemed to be a maximum.

Intervening aliquot amounts indicated a steadily increased percentage yield with decreasing volume used.

It was therefore evident that the method was entirely inadequate for such large amounts of lactic acid. The older method of Friedemann and Kendall *(17)* using a higher concentration of MnO_2, oxidizing agent and larger amounts of manganese sulfate and phosphoric acid in the distillation flasks was found satisfactory. The oxidizing agent was 0.08 N MnO_2, suspension. Distillation was started with approximately 200 ml. volume in a 500 ml. Kjeldahl flask. The manganese sulfate-phosphoric acid reagent was made so that 10 ml. of it would make the distillation mixture one-percent manganese sulfate and 0.1 molar phosphoric acid. The reagent contained 225 g. $MnSO_4 \cdot H_2O$ and 140 ml. 85-percent phosphoric acid per liter. Approximately 150 ml. distillate was collected. Yield was proportional to volume of extract prepared as below at least up to 40 ml. A sample of 20 ml. was found satisfactory with 0.004 N iodine for titration.

For combined lactic and pyruvic acid procedure, five g. tissue was extracted by centrifugation with boiling water three times in 50-ml. tubes. The hot, extract was poured through a filter to separate most of the fat. To the extract was added five ml. 20-percent copper sulfate solution and five ml. 20-percent calcium hydroxide suspension. After standing at least one-half hour with occasional shaking, the precipitate was removed and washed with water three times by centrifugation. The extract was made up to 250 ml., and 20-ml. aliquots were taken for lactic and pyruvic acid determinations.

The pyruvic acid sample was reduced with one-half g. zinc and 2.5 g. sodium acid sulfate over night on a steam bath by the method of Kendall and Friedemann *(26)*. After neutralization to litmus the precipitated zinc hydroxide was removed and washed by centrifugation; the entire extract was analyzed for lactic acid as before. It was found by use of pure lactic and pyruvic acid standards that 95-percent recovery was obtained for lactic acid, and 95 percent of pyruvic acid was converted to lactic acid.

By this method 870 mg. lactic acid per 100 g. wet muscle tissue was found in a sample of fresh, unfrozen beef, and 888 in another. After 234 mg. lactic acid had been added to the first meat, a total of 1130 mg. was obtained by analysis, in error by 2.3 percent.

A bright sample (pH 5.44) of beef rib eye (held in frozen storage 11 months) contained 989 mg. lactic acid. A dark sample (pH 6.24) under same storage contained 613 mg. lactic acid, This difference in acid content of the two samples was approximately sufficient to account for the 0.8 pH unit difference in their acidity, or 470 mg. lactic acid per unit pH per 100 g. of tissue.

A trace of pyruvic acid was found in two fresh, unfrozen samples of bright beef, six mg. in one and nine mg. per 100 g. in the other. No pyruvic acid was found in two dark samples and four bright samples of beef rib eye, all of which had been held in frozen storage 11 months.

LACTIC ACID GENERATION

The foregoing observations point to the conclusion that the difference in amount of lactic acid in bright and dark beef is of primary importance and significance, and that it may be the factor upon which depends most, if not all, the characteristic differences between bright and dark beef.

Consequently experiments on glucose and glycogen conversion were repeated with particular attention paid to lactic acid changes, making use of the revised technic of analysis.

A summary of the results appears in Table 44. It was apparent that addition of glucose had little, if any, effect on lactic acid changes in either the dark or bright sample during incubation. The wide difference in the two samples incubated without added glucose (Table 44, third items in series 1 and 2) may be attributed to their difference in content of native glycogen discussed below.

Likewise, addition of glycogen to the bright sample (Table 44, series 3) had practically no influence on lactic acid changes during incubation. Lactic acid was generated, however, during incubation, as shown in Table 44, series 3, item 2. More than sufficient native glycogen was present (Table 45) to account for the increase in lactic acid. This fact probably explains why additional glycogen had practically no effect in generating more lactic acid. The significant fact remains that the tissue retained its ability to produce lactic acid, and it contained ample native glycogen for that purpose.

Significant increases in lactic acid appeared when glycogen was added to the incubated preparations of the dark sample (Table 44, series 4). Therefore, it was evident that the deficiency of lactic acid in the dark sample was not caused by inhibition of its lactic-acid-producing enzyme system. Rather, it was simply due to glycogen deficiency. When glycogen was supplied, the tissue was able to build up its lactic acid rapidly.

The question now arises whether the accumulation of lactic acid or the drop in pH finally inhibits the further conversion of glycogen to lactic acid in normal bright beef. The fact that the pH of bright beef is consistently near 5.5 suggests that glycogen conversion to lactic acid is automatically stopped when that pH is reached.

However, the effect of adding more lactic acid and adjusting to original pH was first tried as shown in Table 44, series 5 to 8. The same quantity of glycogen was added to the first two preparations of each of the series 5 to 8. After addition of the lactic acid, the pH was adjusted to 6.38 with 0.75 N NaOH.

The first item in each of series 5 to 8 indicated no significant change in lactic acid was brought about by simply adding the glycogen and extracting immediately with hot water. The apparent losses in added glycogen, under similar immediate extraction procedure previously discussed under glycogen conversion, do not seem therefore, to have been attributable to lactic acid formation in the brief period of time involved in the glycogen loss.

TABLE 44.—LACTIC ACID GENERATION. EFFECT OF GLUCOSE, GLYCOGEN, AND LACTIC ACID ADDED TO AQUEOUS DISPERSIONS OF BRIGHT AND DARK BEEF RIB EYE ON LACTIC ACID CHANGES DURING INCUBATION AT 38° C.

SAMPLE NUMBER	Normal pH	Incubation pH	Incubation period	Material added per 100 g. rib eye†	Lactic acid change per 100 g. rib eye‡
			Hours	*Mg.*	*Mg.*
Series 1			0*	A200	— 13
			0	A400	+ 6
W21 (bright)	5.68	6.38	6	0	+ 21
			6	A200	— 15
			6	A400	— 7
Series 2			0	A200	— 2
			0	A400	— 4
W23 (dark)	6.38	6.38	6	0	— 43
			6	A200	+ 15
			6	A400	— 6
Series 3			0	B254	— 8
			6	0	+ 42
W21 (bright)	5.68	6.38	6	B127	— 2
			6	B254	0
			6	B381	— 10
Series 4			0	B254	— 2
			6	0	— 4
W23 (dark)	6.38	6.38	6	B254	+106
			6	B381	+103
			6	B508	+ 86
Series 5			0	B254,C 0	— 2
W23	6.38	6.38	6	B254,C 0	+112
			6	B 0,C 0	— 12
Series 6			0	B254,C190	— 14
			6	B254,C190	+ 46
W23	6.38	6.38	6	B 0,C190	— 35
			0	B 0,C190	— 4
Series 7			0	B254,C380	+ 1
			6	B254,C380	+ 73
W23	6.38	6.38	6	B 0,C380	— 17
			0	B 0,C380	— 33
Series 8			0	B254,C570	+ 9
			6	B254,C570	+ 73
W23	6.38	6.38	6	B 0,C570	— 6
			0	B 0,C570	— 69
Series 9		6.38	6	B254	+ 49
		6.00	6	B254	— 30
W23	6.38	5.45	6	B254	— 23
Series 10		6.03	6	B254	+ 46
W18	6.03	6.89	6	B254	+ 49

* Zero incubation time. Duplicate sets of preparations immediately analyzed (one set containing the added material shown in column 5) are designated by zero incubation time. The difference in lactic acid content of the two sets of preparations is shown in column 6, and it represents change produced by adding the material without incubation. In series 5 to 8 where no glycogen was added, it represents the difference between lactic acid obtained by immediate analysis of preparations and the sum of native lactic acid plus the amount of lactic acid added.

† Material added: A, glucose; B, glycogen; C, lactic acid.

‡ Change in lactic acid corresponding to material added in column 5 represents difference in lactic acid content between two sets of preparations, one containing the added material, and both analyzed after simultaneous incubation.

Change in lactic acid corresponding to no added material represents change in lactic acid during incubation and was obtained by difference between duplicate sets of preparations, one analysed immediately and the other after incubation. The same procedure was followed in series 5 to 8 where no glycogen was added. When glycogen was added in series 5 to 8, it was treated as the only variable, and the same amount of lactic acid was added to both sets of preparations to be incubated, but glycogen was added to only one set.

TABLE 45.—ANALYSES OF SAMPLES USED IN TABLE 44. CONTENTS PER 100 G. RIB EYE

SAMPLE NUMBER	Lactic acid	Glucose	Glycogen	Moisture	Ash
	Mg.	*Mg.*	*Mg.*	*Grams*	*Grams*
W18	628	.....	2	74.76	1.10
W21	722	79	64	69.36	0.98
W23	490	31	2	70.27	1.01

The second item in each of series 5 to 8 indicated significant increases in lactic acid formed from the added glycogen. The added lactic acid appeared to impede to some extent the generation of more lactic acid, but not in proportion to the amounts added.

The third item in each of series 5 to 8 indicated losses in lactic acid occurring during incubation when no glycogen was added. These losses appeared to decrease with increasing amounts of added lactic acid, but that behavior was probably an illusion caused by the tendency of lactic acid to polymerize in aqueous solutions.

The stock solution of lactic acid added to the preparations in series 6 to 8 was made by dissolving two ml. 85-percent lactic acid in one l. of water. By the oxidation method the solution was found to contain 1.57 mg. per ml., but when it was added to the dispersed tissue preparations, the amount recovered far exceeded expectations on that basis. An aliquot of the lactic acid solution was then heated one hour on a steam bath with an excess of 0.1 N NaOH and back-titrated with 0.05 N potassium acid phthalate. The concentration of lactic acid thus analyzed was found to be 2.36 mg. per ml. This concentration can not be interpreted rigidly to indicate the concentration of the original stock acid, because the two-ml. pipette containing syrupy lactic acid was rinsed into the diluted solution.

Therefore, the concentration of the lactic acid solution added in series 6 to 8 was determined by the NaOH titration method and represented concentration of lactic acid after depolymerization. In series 6, item 4, the slight change in lactic acid during immediate extraction indicated that depolymerization must have been completely accomplished in the process. But in item 4 of series 7 and 8, increasing losses were noted, indicating that depolymerization of these larger amounts of added lactic acid had not been accomplished in the short time interval involved. But depolymerization probably was accomplished during the six-hour incubation period in item 3, series 6 to 8. Since lactic acid changes in this item were based on analyses of duplicate preparations, one immediately after mixing, and the other after incubation, it is evident that losses during incubation would be counteracted by release of more lactic acid by depolymerization. The evidence thus strongly indicated that the decrease in losses of lactic acid in item 3 of series 5 to 6 was not caused by an inhibiting effect of increasing amounts of added lactic acid, but rather it was caused by an increase of monomolecular lactic acid through depolymerization of the added lactic acid during incubation.

The effect of increased acidity on formation of lactic acid from glycogen is shown in Table 44, series 9. The pH changes were induced by adding 0.68 N HC1 to the aqueous dispersions. The evidence mas convincing that increase of acidity effectively inhibited conversion of glycogen to lactic acid, and it actually appeared to effect losses rather than gains in lactic acid during incubation with added glycogen.

The question as to whether lactic acid may have been converted back to glycogen under the more acid environment is to be investigated later. According to Diemair and Mollenkopf *(12)*, animal cells under certain conditions can convert sugar to lactic acid, which is to a considerable extent resynthesized into glycogen, and following death the rapid rise in lactic acid roughly corresponds to the decrease in glycogen.

The effect of decreased acidity on formation of lactic acid from glycogen is shown in Table 44, series 10. The pH was increased by adding 0.72 N NaOH to the aqueous dispersions. A corresponding increase in generation of lactic acid from the added glycogen gave further convincing evidence that pH characteristic of black beef promoted rapid conversion of glycogen to lactic acid, whereas that conversion previously had been shown definitely to be inhibited by pH characteristic of normal bright beef.

In conclusion, the cause of dark-cutting beef definitely seems to rest upon a deficiency of glycogen in the tissue at the time of slaughter. Many factors may be involved in the quantity and stability of glycogen in the animal tissue beside simply the feed the animal gets. In a survey made by the Meat Board Committee *(39)* on occurrence of dark cutters, it was found that calves yielding dark beef received on the average 37 percent more roughage than calves yielding bright beef, a higher percentage of them were nervous, and a higher percentage of them had been castrated after the age of three months. The results were based on 58 calves in each group.

Nervousness naturally suggests susceptibility to shock, and it is quite possible that glycogen, as well as glucose, is thrown out of animal tissues by shock. The question of conditioning of animals to withstand shock was discussed in the beginning of Part IV. It is planned to investigate the quantity and stability of glycogen in grass-fed and in grain-fed beef animals.

Winter and Thomson *(51)* disagree with other investigators who found that castration reduced muscle glycogen in male rats. The age at castration may have been an unreckoned variable in this, controversial subject. However, Winter and Thomson agreed that low levels of muscle glycogen were found in rats affected by adrenal insufficiency.

Therefore, it seems probable that glycogen deficiency in muscle tissue may result from a number of unrelated causes, and that extensive investigations will be necessary to determine all the causes upon which may depend the occurrence of dark-cutting beef.

(For summary and conclusions, see page 79.)

SUMMARY AND CONCLUSIONS

PART I. MINERAL CONSTITUENTS

Samples of blood from 16 animals were analyzed for phosphorus, calcium, sodium, potassium, and chlorine. Muscle tissue from these same animals was analyzed for water, fat, protein, ash, phosphorus, calcium, magnesium, sodium, and potassium.

The adipose tissue from these animals was analyzed for water, fat, protein, ash, phosphorus, calcium, sodium and potassium. Five of these animals were full-fed in dry lot, five were full-fed on pasture, two were grazed on grass of normal mineral composition, two were grazed on grass of low-phosphorus content, and two were grazed on grass of low-phosphorus content but were given limited quantities of bone meal. The muscle tissue from two samples of meat from animals commonly known as dark-cutters, the method of feeding not known, was analyzed for water, fat, protein, ash, phosphorus, calcium, magnesium, sodium, and potassium.

Ripening shrinkage was found to be dependent upon mineral constituents as well as upon fat covering.

The influence of the ratios of the mineral constituents upon the keeping quality of the meat was discussed. No correlation could be detected between the total mineral constituents of meat and its keeping quality, except to a limited extent in the adipose tissue. Here the low-phosphorus condition was most apparent in the low P/N ratios in the lots on grass alone as compared with the lots receiving corn.

The effect of phosphorus intake on calcium retention was reflected in the relatively high Ca/N ratios in the fat tissue of corn-fed animals.

The poor keeping quality of meat from animals receiving low-phosphorus grass, as compared to meat from animals receiving grass of normal-phosphorus content, indicated that this element in the ration had an important relation to the keeping quality of the meat produced.

PART II. PHOSPHORUS DEFICIENCY

Ten yearling steers were fed by the paired-feeding method on high and low levels of phosphorus. Steers on low phosphorus developed definite symptoms of phosphorus deficiency as shown by blood and bone analyses.

Rib roasts from the high-phosphorus steers were judged higher grade in tenderness, desirability of flavor of lean, quality of juiciness, desirability of aroma, intensity of flavor of fat, and texture.

After ripening 28 days, rib cuts from the high-phosphorus steers suffered less spoilage and lower shrinkage losses than the low-phosphorus cuts.

High-phosphorus roasts required less cooking time per pound, but cooking evaporation losses were higher.

Mechanical-shear measurement of tenderness and collagen content indicated no pronounced distinction in tenderness between high- and low-phosphorus samples, suggesting that palatability grade for tenderness may have been influenced by other superior palatability factors for high-phosphorus beef.

A method was described for pressing the fluid from raw or cooked muscle tissue using a Carver laboratory press.

Higher quantity of press fluid was obtained from the low-phosphorus rib-eye samples, both raw and cooked.

A higher yield of calcium was obtained in the press fluid of the low-phosphorus samples in spite of the fact that the whole tissue contained less total calcium than the high-phosphorus samples. Higher retention of calcium in high-phosphorus rib eye was thus clearly indicated.

No significant color differences were observed in the samples and no shady or dark colored specimens appeared. However, a slight tendency toward dark beef characteristics was apparent in higher pH values and lower oxidation potentials in low-phosphorus samples.

Fat tissue appeared to be more profoundly affected than muscle tissue by phosphorus deficiency. Lower P/N and Ca/N, and higher P/Ca ratios were found to be characteristic of low-phosphorus fat tissue.

In conclusion, phosphorus-deficient beef was inferior to high-phosphorus beef in palatability, keeping quality, and shrinkage loss.

PART III. LIMESTONE SUPPLEMENT

Twelve yearling steers were fed by the paired-feeding method, a ration of corn, cottonseed meal, and silage; one member of each pair received 0.1 lb. ground limestone daily.

Only insignificant differences were found in blood calcium and blood phosphorus. But in the bones higher ash, phosphorus, calcium, and much higher breaking strength and specific gravity were found in the limestone lot.

No palatability distinction was found between rib roasts from the two lots, except slightly lower tenderness for the limestone lot.

After ripening 21 days, rib cuts from the limestone lot appeared to have suffered slightly less spoilage, but shrinkage losses appeared largely dependent on outside fat covering.

Cooking time and cooking evaporation losses likewise appeared dependent almost entirely on outside fat covering.

Mechanical shear test for tenderness indicated no distinction between lots.

Slightly more collagen was found in the limestone lot. Collagen was found to be 21 percent of the water-insoluble fraction of rib-eye protein.

No distinction was observed between lots in quantity of press fluid obtained from the rib eye, nor was any distinction observed in regard to phosphorus-calcium relations in the rib eye or its press fluid.

A trend toward darker color was found in rib eyes of the limestone lot.

No distinction was observed between lots in regard to gross composition or phosphorus-calcium-nitrogen relationships in outside rib-fat tissue.

In conclusion, only slight and insignificant differences were found in the quality of beef from steers fed on high and low levels of calcium. Although distinct differences were observed in gains in weight and bone strength, no distinction was observed in calcium mineralization of tissues. These findings were in contrast with the significant changes in both phosphorus and calcium in tissues when steers were fed on high and low levels of phosphorus.

PART IV. DARK-CUTTING BEEF

Early investigations on dark-cutting beef were discussed.

Characteristics of dark-cutting beef muscle tissue are abnormally high pH, low glucose, practically no glycogen, high inorganic phosphate, low oxidation potential, and rapid oxygen uptake.

In these investigations it was shown that colloidal behavior and degree of water imbibition of black beef and bright beef muscle tissue were practically the same at the same pH.

Black beef and bright beef muscle proteins had practically the same isoelectric point.

In a steer receiving only water for the 10 days preceding slaughter, blood glucose reached a minimum on the third day, and muscle glucose reached a minimum on the sixth day. Muscle glucose continued to rise six days after slaughter, when it was nearly normal for bright beef.

Immediately following slaughter of a full-fed steer, periodic determinations indicated rapid changes during the first 12 hours followed by stable values in pH, glycogen, lactic acid, pyruvic acid, and inorganic phosphorus. Glucose continued to rise eight days.

Added glycogen was converted more rapidly in dark beef than in bright beef. Native glycogen was converted more rapidly than added glycogen in bright beef.

Added glucose was converted during incubation much more rapidly in black beef than in bright beef. At the same time inorganic phosphate increased 50 percent in the dark sample and remained unchanged in the bright sample.

No significant difference was found in the buffer strength of bright beef and dark beef. Hydrogen ion concentrations were reduced to approximately 1/15,000 of original after dispersion with muscle tissue.

A method was adapted for determining large amounts of lactic acid and pyruvic acid in beef muscle tissue. Approximately 900 mg. lactic acid was found in 100 g. bright beef rib eye having pH 5.50.

Approximately 450 mg. lactic acid or pyruvic acid was required to change 100 g. beef rib eye from pH 6.50 to pH 5.50.

The most significant difference in properties of dark beef and bright beef appeared to be lower amounts of lactic acid in dark beef.

No significant changes in lactic acid occurred during incubation of aqueous dispersions of either dark or bright beef to which glucose was added.

Significant increases in lactic acid occurred in dark beef dispersions incubated with added glycogen.

Conversion of glycogen to lactic acid in dark beef was found to be sharply inhibited by lowering the pH to 5.5, which is characteristic of bright, fresh beef.

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