

amounts of casein likely to be obtained from 50 cc. of milk, and provided the precipitate is allowed to drain for a sufficient time this error is, for all practical purposes, negligible. It could, of course, be eliminated, if desired by diluting the final solution, before filtration, to an accurately measured volume with $N/10$ NaOH; in that case it would be advisable to start with 100 cc. of milk instead of 50 cc., completely dissolve the precipitate in 100 cc. of $N/10$ NaOH and then make up the volume of the mixture to 200 cc. by the addition of $N/10$ NaOH. The slight dilution of the *sodium hydroxide* caused by this procedure does not affect the accuracy of the determination, since the refractive index of a dilute sodium hydrate solution varies very much less with its concentration than that of a solution of casein of the concentrations employed above.

For comparison, determinations were carried out by the official method¹ as follows:

Twenty cc. of fresh unskimmed milk were diluted to 100 cc. and 30 cc. of $N/10$ acetic acid were slowly added, the mixture being continuously and rapidly stirred during the addition. The precipitate was then allowed to settle and the supernatant fluid was poured through a 15 cm. S. & S. No. 589 "white band" paper. The precipitate was then washed by decantation with distilled water several times, the washings, and, subsequently, the precipitate being transferred to the same paper. The filter and precipitate were then digested with 20 cc. of H_2SO_4 , to which a trace of metallic mercury had been added, as in the ordinary Kjeldahl method for the determination of nitrogen, and the process of determining the nitrogen was completed by the official Kjeldahl method. The number of grams of casein in the 20 cc. of milk was estimated by multiplying the number of grams of nitrogen thus determined by 6.25.

Four determinations by the new method yielded the following results:

	Grams casein in 100 cc. milk.
a.....	2.84
b.....	2.84
c.....	2.84
d.....	2.84
Average.....	2.84

Four determinations by the official method,

¹ U. S. Department of Agriculture, Division of Chemistry, Bulletin 46, Revised Edition (1899), p. 55. The details of the precipitation were slightly modified and the quantity of milk employed in the determination was double that recommended by the Association of Official Agricultural Chemists.

using the same milk, yielded the following results:

	Grams casein in 100 cc. milk.
a.....	2.69
b.....	2.81
c.....	2.78
d.....	2.69
Average.....	2.74

The agreement is sufficiently satisfactory. The factor 6.25 by which the nitrogen is multiplied to obtain the equivalent in casein is calculated on the assumption that the percentage of nitrogen in casein is 16. If, however, we take 15.65 as the true percentage of nitrogen in casein, which, according to the results of Hammersten,¹ Lehmann and Hempel,² and Ellenberger,³ would appear to be the more accurate figure, then the factor by which nitrogen is multiplied becomes 6.4 and the agreement between the official and the new methods is even more satisfactory, the above four determinations by the official method yielding the results:

	Grams casein in 100 cc. milk.
a.....	2.75
b.....	2.88
c.....	2.85
d.....	2.75
Average.....	2.81

In order to further test the accuracy of the method, weighed amounts of casein were dissolved each in 100 cc. of $N/50$ NaOH and were precipitated, with constant stirring, by the addition of 30 cc. of $N/10$ acetic acid. The casein employed in these experiments was the c. p. product manufactured by Eimer & Amend and further purified by trituration with large volumes of distilled water alcohol (absolute) and ether (ueber natrium dist.); it was dried for 24 hours at 36° . The properties of the product thus obtained have been fully described by me in a previous paper;⁴ it gives every indication of being a pure product, being insoluble in distilled water (save in traces which adhere to the undissolved particles) and completely precipitated by acetic acid. It neutralizes to phenolphthalein exactly the quantity of base determined by Laqueur and Sackur and by Van Slyke and Hart;⁵ it is free from appreciable water. The first

¹ O. Hammersten, *Zeitschr. f. Physiol. Chem.*, **7**, 227 (1883); **9**, 273 (1885).

² W. Hempel, *Arch. f. d. ges. Physiol.*, **56**, 558 (1894).

³ Ellenberger, *Arch. f. Anat. und Physiol., Physiol. Abt. Suppl.*, p. 313 (1902).

⁴ T. Brailsford Robertson, *Journ. of Biol. Chem.*, **2**, 317 (1907).

⁵ Laqueur and Sackur, *Beitr. z. Chem. Physiol. u. Path.*, **3**, 193 (1902). Van Slyke and Hart, *Amer. Chem. Journ.*, **33**, 461 (1905).

two determinations were carried out in exactly the same manner as those of the casein in milk, described above. The last three, in which larger quantities of casein were determined, were determined in the same manner save that the final solution, before filtration, was diluted to 250 cc. (accurately measured) so as to eliminate the error due to water associated with the precipitate and paper. The results follow:

Grams casein in 100 cc. solution.	
Weighed.	Determined.
1.0	1.02
2.0	1.88
3.0	2.90
4.0	3.88
5.0	5.03

The agreement is as close as could be desired.

If the refractive index of the final solution be determined at temperatures above 20° and below 30°, 0.0001 must be subtracted from the value of the constant n_1 ($= 1.33444$ at 20° when the solvent is $N/10$ NaOH) for every degree by which the temperature exceeds 20°. If it be determined at temperatures below 20° and above 10° 0.00007 must be added to the value of the constant n_1 for every degree by which the temperature is less than 20°.¹

The advantages of the method herein described over the official method for the determination of casein are manifest. Not only is the time consumed in the determination much reduced, the time actually occupied in manipulation, exclusive of that allowed for drainage of the precipitate, being not much over 1/2 hour, but the whole procedure is much simplified and the accuracy of the determination is unimpaired.

For further details concerning the dependence of the refractive index of casein solutions upon their concentration, the nature of the solvent, the temperature, etc., I must refer the reader to my previous paper, cited above.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY,
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CHANGES IN THE COMPOSITION OF THE SKELETON OF BEEF ANIMALS.

(FIRST PAPER.)

By P. F. TROWBRIDGE AND F. W. WOODMAN.

Received July 1, 1909.

The study of the composition of the skeletons of beef animals reported in this paper constitutes a portion of the general study of the "Uses to which

the Animal Puts Its Food," now in progress in the Experiment Station of the University of Missouri. In the work during the past two years, fifteen animals have been slaughtered and analyzed. Of these the six mentioned in this paper were used chiefly to study the resorption of fat which takes place when an animal in good condition of nourishment is unable to obtain a usual food supply. Only the skeleton is considered in this paper.

The housekeeper regards the skeleton as merely so much waste material. To the animal husbandman it is of very great importance, determining, to a large extent, the size and value of the animal. To him an exact knowledge of the conditions of growth of the skeleton and to what extent it is affected by adverse conditions is very important.

Lawes and Gilbert, in their classic researches at Rothamsted, made a special study of the ash content of the carcasses of bees and other animals. They pointed out especially the variation of the proportion of mineral matter in the bones to that in all other parts of the body, dependent upon the degree of fatness of the animal.

Wildt,¹ working with rabbits, made a special study of the effect of age on the chemical composition of the skeleton of young and of full-grown animals. He concluded that age had little effect after the animal had reached full growth, the composition of the skeleton varying with the condition of the animal. Mann² drew the same conclusions, though from rather indefinite data.

During³ gives tables showing the difference in composition of the bones of various parts of birds. He showed that the composition of the air-dry bone varies in different parts of the skeleton.

Brookman⁴ made comparative analyses of the skeletons of cow, sheep, horse and swine.

Gabriel⁵ studied especially the methods of analysis of bones. His investigation of the composition of the bone ash was very thorough and he gives a probable composition of bone ash.

The phosphorus in the marrow is largely in organic form, according to Otolsky.⁶ He found 0.13 per cent. to 0.15 per cent. lecithin in the marrow of the leg bones of the horse. W. Gliken⁷ obtained similar results with both human and animal skele-

¹ *Landw. Versuchsst.*, **15**, 404 (1872).

² *Chem. News*, **51**, 132 (1887).

³ *Zeit. physiol. Chem.*, **7**, 446 (1883).

⁴ *Ber.*, 388 (1882).

⁵ *Zeit. physiol. Chem.*, **19**, 257 (1894).

⁶ *Abs. in Jahresb. u. Tier. Chem.*, **37** (1908).

⁷ *Biochem. Ztschr.*, **4**, 235 (1908).

¹ Cf. my previous paper, referred to above.

tons. He also found that the per cent. of lecithin in the marrow decreases with age.

The effect of nourishment upon the skeleton has been studied extensively, the investigations following three general lines: (1) the effect of feeding rations normal in other respects, but poor in one or more necessary mineral constituents; (2) the comparative effect of organic and inorganic compounds as sources of phosphorus; (3) the effect of starvation upon the composition and weight of the skeleton. The results of the various investigators¹ seems to show: Foods poor in calcium have little effect upon the skeleton. Foods low in phosphorus have a pronounced effect, producing weakness, brittleness and actual loss in weight of the skeleton.

Whether the form of combination of the phosphorus has any effect is still disputed.

Inanition decreases the fat and increases the moisture in the skeleton but does not appear to effect the mineral composition to any noticeable extent.

EXPERIMENTAL PART.

Objects of this Investigation.

In the investigations noted above the variations in the kind and conditions of the animals used, the object and methods of the work, and the varying manner of reporting make it hard to compare the results. In the course of the series of experiments upon the effect of breed, feed and age upon the composition of animal flesh, and the distribution of the food materials in the various parts of the animal body, which investigations are being carried on at the Missouri Agricultural Experiment Station, it became desirable to know the composition of the skeleton of the animals under consideration. With this object in view, the following work was undertaken, having special reference to the distribution of phosphorus in the different parts of the skeleton.

Condition and Age of Steers at Time of Slaughtering.

The six steers reported in this paper were all from the group known as "special maintenance steers." They were from the same sire and herd of cattle, grade Herefords; of about the same age,

spring calves of 1907, and had run on grass until purchased for the experiment in fall of 1907. At the outset all were put upon full feed, consisting of 2.5 parts grain (composed of 8 parts corn to 1 linseed meal) to one part alfalfa hay, until in nearly prime condition.

In February, 1908, steer 594 was killed as a check animal. He was a fat yearling, showing Shorthorn blood, and was in a nearly finished condition. His carcass graded as "baby beef."

Steers 591, 593 and 597 were killed in September, 1908, after having been kept on "special maintenance" for six months.

Steer 591 was eighteen months old when killed, and had been kept on "submaintenance" for six months, being made to lose one-half pound daily. He was very thin, his carcass being graded as a "canner."

Steer 597 was eighteen months old when slaughtered. He had been kept on "maintenance," being kept at the same weight, neither gaining or losing. His carcass graded as No. 3 beef, being too thin for No. 2.

Steer 593 had been kept on "supermaintenance" for six months, being made to gain one-half pound per day. He was eighteen months old when slaughtered and graded as No. 2 beef.

Steer 592 was killed in January, 1909, being about 22 months old when killed. He had been kept on "submaintenance" for eleven months, was exceedingly thin and graded as a "poor canner." The appearance of the skeleton of this steer was remarkable, the marrow having nearly all disappeared, and in its place was a watery, ill-smelling liquid, having none of the ordinary properties of normal marrow, and no greasy or fatty appearance whatever.

Steer 595 was slaughtered in February, 1909, after having been kept on "maintenance" for a year. He was graded as a "canner."

The feed of all these animals was exactly the same in quality, differing only in quantity from the beginning, so that whatever differences appear in the skeleton are due to amount of food and individuality of the animals.

Method of Obtaining Samples.

The samples were obtained at the time of slaughtering and when the carcass was cut up, forty-eight hours later. The carcass of each animal was cut into the regular wholesale cuts, and the right

¹ Weiske, *Zeit. physiol. Chem.*, **20**, 595 (1895). Lehman, *Jahresb. u. Agr. Chem.*, **20**, 382 (1877). Sedlmair, *Zeit. f. Biol.*, **37**, 25 (1898). Gusmita, *Jahresb. u. Thier. Chem.*, **1884**, 401. Forbes, *Bull.* **201** (1909). Ohio Agr. Expt. Station. Wiley, "Composition of Carcass of Pigs," *U. S. Dept. Agr., Division of Chemistry, Bull.* **53**, page 70 (1898). Hart, McCullum and Fuller, *Am. Jour. Physiol.*, **23**, 246 (1909). Hart, McCullum and Humphrey, *Ibid.*, **24**, 86 (1909).

half taken for all chemical analyses, being hand-separated into lean, fat and bone.

The bone samples were made up for analysis as follows: (1) head and tail; (2) feet minus hoofs; (3) shin and shank; (4) chuck and neck; (5) flank and plate; (6) ribs; (7) rump; (8) loin; and (9) round. The reasons for combining the samples in pairs in some cases was to reduce the number of samples.

The whole bone was taken for analysis, including such adhering fat, lean and tendon as could not be removed with a knife. The bones were not boiled nor heated to remove the adhering flesh in any case. The marrow was thus included in the sample.

In the case of 594, the bones of the various cuts were not analyzed separately, but a composite of the whole was made and analyzed as one sample. The analysis of this animal will therefore be comparable only with the averages of the others.

METHODS OF ANALYSIS.

Preparation of Sample for Analysis.

As the samples of bone were removed from the carcass they were weighed quickly to avoid loss of moisture, then broken up, and ground in a Mann's bone grinder. The ground sample was mixed thoroughly and quartered down to the desired size of sample. This portion was put into closed jars and sent at once to the laboratory or kept in the cooler until it could be weighed out for analysis.

Moisture.

Samples for moisture and fat were weighed out rapidly, in triplicate, in tared porcelain evaporating dishes, the size of the sample varying according to the fat content, as judged by the appearance of the bone. For fat, greasy samples, 25-40 grams were considered sufficient while for those with little fat 100 grams or even more were sometimes taken.

The dishes containing the weighed samples were at once placed in vacuum desiccators and dried over sulphuric acid, at room temperature, by means of the Benedict vacuum method, as modified for use in this laboratory.¹

This method has proved very satisfactory, agreeing very closely with the older methods, and leaving the sample in perfect conditions for further analyses.

¹ P. F. Trowbridge (Nov., 1908), Proceedings of the Association of Official Agricultural Chemists (*U. S. Dept. of Agriculture, Bureau of Chemistry, Bull. 122*). L. F. Shackell, *Am. Jour. Physiol.*, **24**, 325 (June, 1909).

Fat.

The determination of fat was made upon the same samples as for moisture. The dry substance was transferred carefully to Soxhlet extractors prepared by placing a pad of absorbent cotton in the bottom to filter the ether extract.

Redistilled anhydrous ether was used for the extraction, the fat being collected in tared flasks and weighed after drying for 24 hours in vacuum.

After drying and extraction the triplicate samples were combined in one and ground in a steel mill until fine enough to pass through a millimeter mesh sieve. This ground, air-dry sample was used for the determination of ash and phosphorus.

Ash.

Two gram samples were weighed out in tared porcelain crucibles dried at 100-110°, and the moisture determined. The samples were ashed by igniting over bunsen burners until practically free from carbon, the ignition being completed in the muffle at dull red heat. A clear, white ash could be readily obtained by this means in a short time.

Phosphorus.

The ash from the above determination was dissolved by digestion in hot, dilute nitric acid and the solution made up to 250 cc. Aliquots of 25 cc. were taken and the phosphorus determined gravimetrically as in fertilizers by the official methods of the Association of Official Agricultural Chemists (*U. S. Dept. Agr., Bureau of Chem., Bull. 107* (revised)).

DISCUSSION OF THE RESULTS.

In an investigation conducted, as this one was, with animals which must of necessity differ in many characteristics, much depends upon selecting animals which are as nearly alike in all respects as it is possible to obtain them. The steers in the "special maintenance" group, of which these six steers were a part, were all selected with the greatest care to obtain animals of the same age, size, breed, and general condition. However, even after this careful selection there were individual characteristics which influenced the results, especially the weight and proportion of the skeleton.

The weights of the skeleton as shown in Tables I and II are almost solely a matter of the individual characteristics of the animals. No. 594, killed at the outset, was the smallest of all the steers and the total weight of his skeleton is also the smallest. Measurements made during the feeding period show that the special maintenance steers all con-

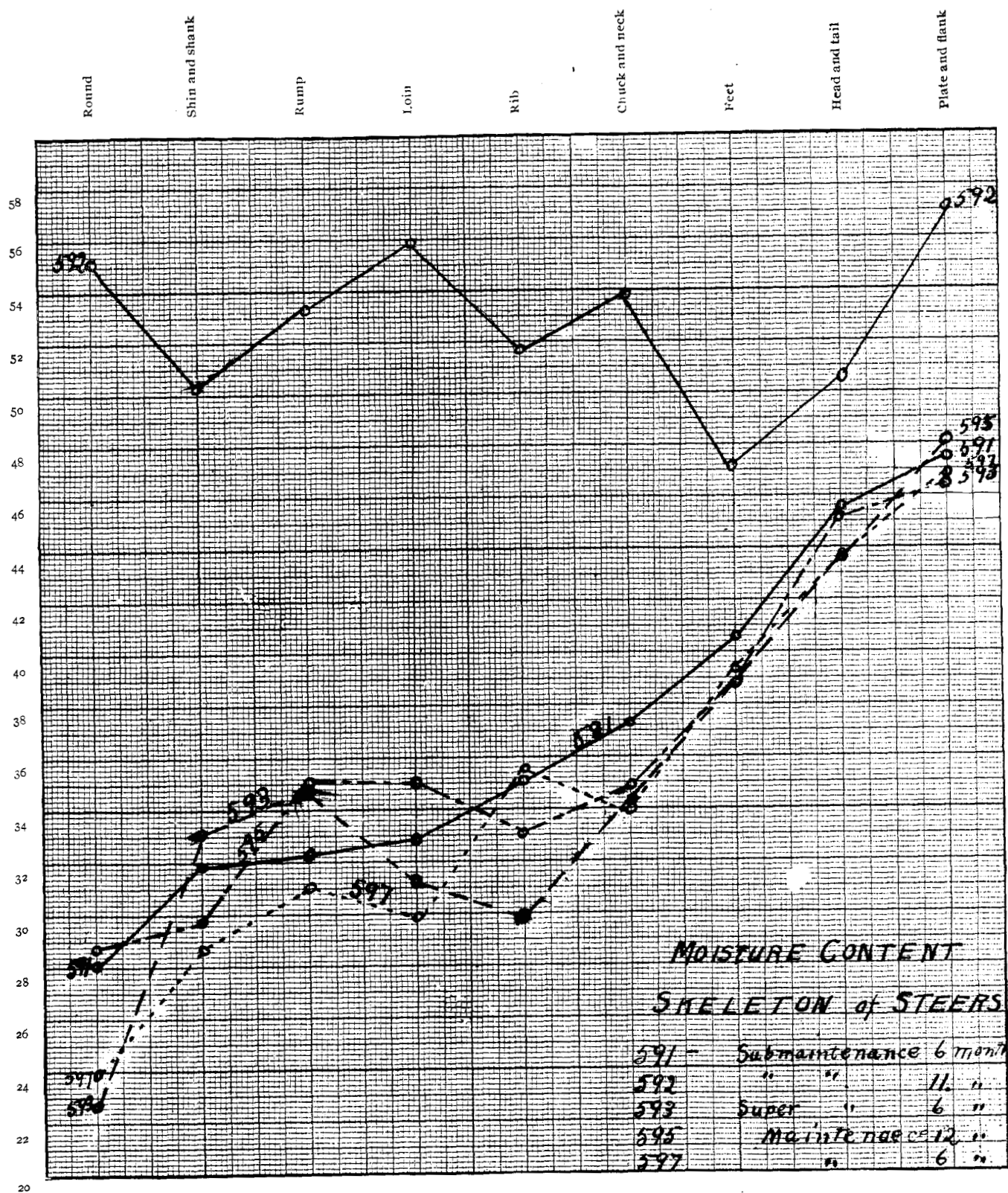


Plate 1.

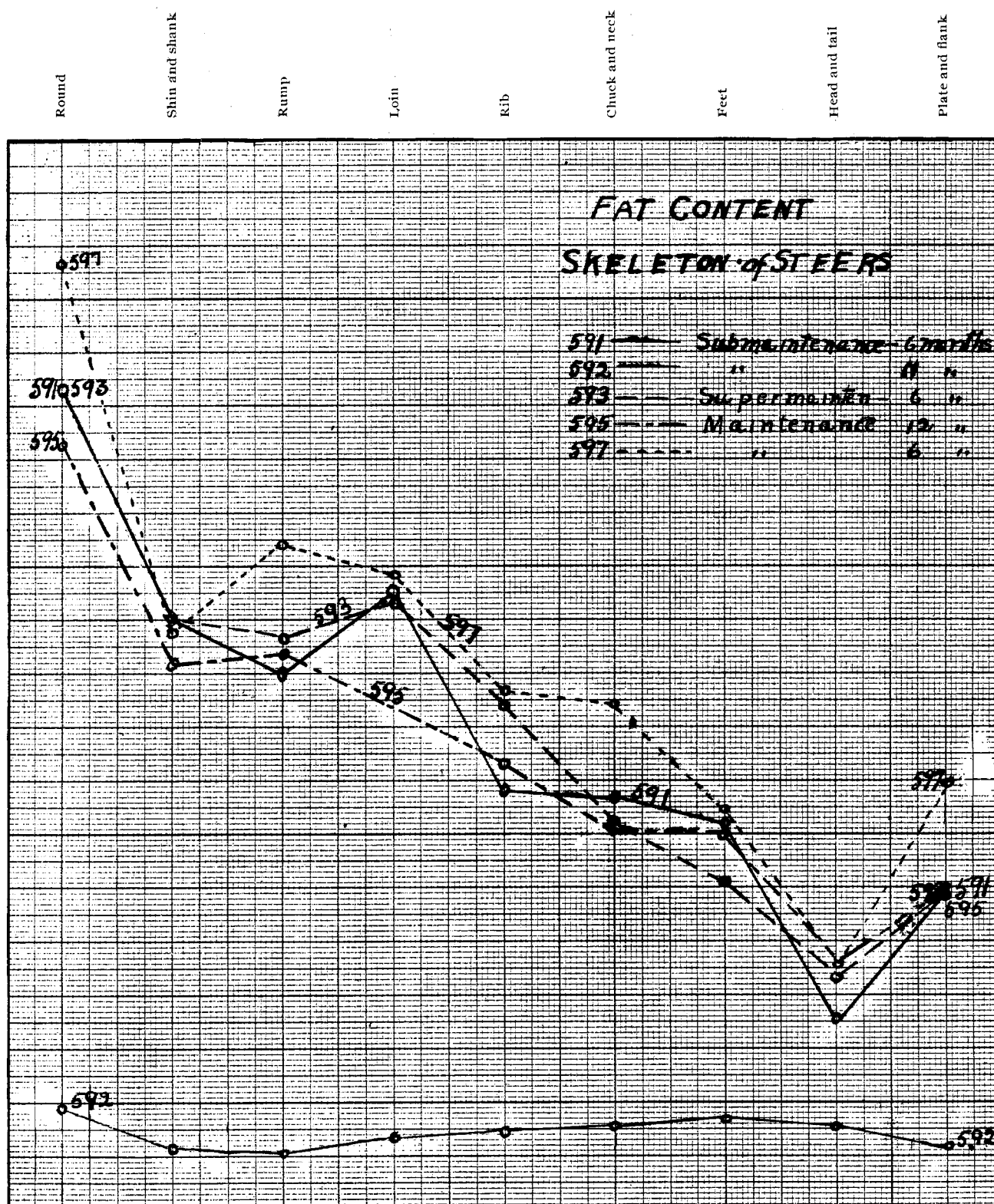


Plate 2.

The sum of the per cents. of moisture and fat is, however, nearly the same for the composite of the whole skeleton in all of the animals as shown in Table I. The fact that in steer 592 the sum of the moisture and fat in the skeleton is a little higher than in the other steers indicates that when fat is resorbed water is deposited in its place, and the re-

same for the different animals, the per cent. of ash in the original sample will be of little value, since it will be entirely dependent upon the sum of fat and moisture.

The per cent. of ash in the original sample varied with no regularity in the separate cuts of the individual animals. The only cuts in which all the

TABLE I.
SUMMARY OF COMPOSITION OF SKELETON OF SIX SPECIAL MAINTENANCE STEERS.

	Weight of skele- ton, kilos.	Empty weight of steers, kilos.	Per cent. skeleton to empty weight.	Moisture in skele- ton. Per cent.	Ether-soluble (fat) in skeleton. Per cent.	Sum of moisture and fat in skele- ton. Per cent.	Ash in skeleton Per cent.	Phosphorus in ash of skeleton. Per cent.	Phosphorus in skeleton. Per cent.	Ash in skeleton freed from mois- ture and fat. Per cent.	Phosphorus in skeleton freed from moisture and fat. Per cent.	Organic matter in skeleton not fat. Per cent.	Remarks.
Steer No. 594	39.270	247.52	15.86	39.89	14.68	54.57	23.81	18.48	4.40	52.40	9.11	21.63	Slaughtered as check animal, Feb., 1908
Steer No. 593	51.104	317.92	16.07	35.36	17.63	52.99	25.29	18.15	4.59	53.81	9.77	21.72	Six months supermaintenance, slaughtered, Sept., 1908
Steer No. 597	51.012	302.56	16.86	34.32	19.53	53.85	24.72	18.65	4.61	53.57	9.99	21.43	Six months maintenance, slaughtered, Sept., 1908
Steer No. 595	44.912	230.24	19.51	36.11	16.47	52.58	26.42	18.89	4.99	55.72	10.53	21.00	One year maintenance, slaughtered, Feb., 1909
Steer No. 591	40.396	190.31	21.22	37.02	17.32	54.34	23.81	18.45	4.39	52.15	9.62	21.85	Six months submaintenance, slaughtered, Sept., 1908
Steer No. 592	48.430	190.20	25.46	52.46	3.09	55.55	22.76	18.29	4.16	51.19	9.37	21.68	Eleven months submaintenance, slaughtered, Jan., 1909

verse may very probably be true during the fattening of animals and may explain why a steer may be getting fat but not gaining weight.

Organic Matter not Fat.

The organic matter other than fat was calculated for the individual cuts of each animal, and it seems to be practically constant for all animals, varying in the cuts calculated between the limits 20.5 per cent. and 22.5 per cent. in the different parts of the same steers. For the composite of each of the six animals this content of organic matter, not fat, varied between the limits of 21.00 per cent. and 21.95 per cent., or is practically constant. This was calculated by difference, being the loss on ignition of the dry and fat-free substance. The constant proportion of this organic matter which forms the framework of the skeleton shows that in none of the animals was the ratio of the organic portion other than fat changed by feeding during the period. The slight differences in the per cent. of this organic substance are no greater than can be attributed to the variations in sampling or other causes.

Ash.

The ash represents the mineral portion of the skeleton and is a large part of the total weight (Tables I and V). Since, as has been shown, the organic matter other than fat is practically the

animals were similar were the flank and plate, which was lowest in ash in all cases, and the head and tail which analyzed very close to 25.00 per cent. of ash in all animals.

TABLE II.
WEIGHT OF SKELETON OF THE SEPARATE CUTS, GRAMS.

No. of steer.....	593	597	595	591	592	594
Head.....	6302	6357	5620	4888	4454	4740
Tail.....	222	270	280	146	342	146
Feet.....	5574	5378	4584	4570	5112	4576
Shins.....	4132	4114	3344	3572	4014	2974
Shanks.....	4650	4503	4032	3668	4316	3362
Chucks.....	9259	9820	8006	7248	8906	6890
Neck.....	756	932	952	796	1112	720
Flanks.....	63	32	92	80	76	78
Plates.....	4187	4240	3650	3324	4432	2998
Ribs.....	4325	4382	4290	3378	5050	3696
Loins.....	5001	5172	4690	3905	4446	4654
Rumps.....	2164	1644	1582	1335	1788	1120
Rounds.....	4470	4168	3790	3516	4380	3312
Total.....	51105	51012	44912	40396	48430	39266

TABLE III.
PER CENT. MOISTURE IN SKELETONS OF SPECIAL MAINTENANCE STEERS.

No. of steer.....	593	597	595	591	592	...
Head and tail.....	43.70	43.83	45.12	45.58	50.77	...
Feet.....	38.58	38.66	39.34	40.55	47.29	...
Shins and shanks...	33.31	28.69	29.74	31.94	50.37	...
Chucks and neck....	34.24	34.00	34.90	37.33	53.81	...
Flanks and plates..	48.19	46.99	46.43	47.46	57.21	...
Ribs.....	30.86	35.42	33.01	35.12	55.95	...
Loins.....	31.20	29.92	35.14	32.87	51.67	...
Rumps.....	34.86	31.01	34.93	32.84	53.28	...
Rounds.....	22.71	23.93	28.69	28.10	55.13	...
Total skeleton.....	35.36	34.32	36.11	37.02	52.46	39.86

The per cent. of ash in the dry and fat-free substance (Table VI) is a better indication of the ash content than the per cent. in the fresh sample.

Steer 595, the oldest of the lot when killed, was highest in ash content, this being not true of all parts of the skeleton, but of the skeleton as a whole.

TABLE IV.

PER CENT. FAT IN SKELETONS OF SPECIAL MAINTENANCE STEERS.						
No. of steer.....	593	597	595	591	592	594
Head and tail.....	8.68	9.05	9.14	7.18	3.15	...
Feet.....	12.23	14.82	14.03	14.42	3.43	...
Shins and shanks....	21.92	21.56	20.36	22.03	2.25	...
Chucks and neck....	14.53	18.91	14.13	15.26	3.13	...
Flanks and plates ..	11.87	15.85	11.95	11.68	2.25	...
Ribs.....	18.79	19.31	16.59	15.56	2.69	...
Loins.....	22.92	23.57	18.74	23.10	2.87	...
Rumps.....	21.30	24.76	20.68	20.09	2.15	...
Rounds.....	30.48	35.29	28.62	30.39	3.81	...
Total skeleton.....	17.63	19.53	16.47	17.32	3.09	14.68

TABLE V.

DISTRIBUTION OF ASH IN SKELETONS OF SPECIAL MAINTENANCE STEERS; EXPRESSED IN PER CENT. OF FRESH WT.

No. of steer.....	593	597	595	591	592
Head and tail.....	25.00	25.00	25.61	25.29	25.08
Feet.....	25.32	23.73	25.78	22.14	24.42
Shins and shanks....	24.41	27.81	28.76	24.91	26.28
Chucks and neck....	27.42	25.83	28.05	24.23	21.45
Flanks and plates....	17.24	15.36	19.63	17.71	17.62
Ribs.....	27.86	23.69	27.44	26.17	22.36
Loins.....	24.75	25.81	26.83	23.59	20.66
Rumps.....	25.94	23.66	24.13	26.20	23.57
Rounds.....	28.12	24.01	26.03	23.60	22.30
Total skeleton.....	25.29	24.72	26.42	23.81	22.76

TABLE VI.

PER CENT. OF ASH. REFERRED TO SKELETON FREED FROM MOISTURE AND FAT.

No. of steer.....	593	597	595	591	592	594
Head and tail.....	52.51	53.07	56.00	53.54	54.36	...
Feet.....	51.48	51.00	55.29	49.19	49.56	...
Shins and shanks....	54.54	55.91	57.62	54.11	55.47	...
Chucks and neck....	53.52	54.85	55.03	51.10	49.83	...
Flanks and plates....	43.17	41.35	46.92	43.34	43.47	...
Ribs.....	55.34	52.33	54.46	53.07	49.20	...
Loins.....	53.95	55.58	58.17	53.59	49.95	...
Rumps.....	58.67	53.50	54.37	55.07	52.90	...
Rounds.....	59.95	58.87	60.98	56.85	54.31	...
Total skeleton.....	53.81	53.57	55.72	52.15	51.19	52.40

TABLE VII.

DISTRIBUTION OF PHOSPHORUS IN SKELETONS OF SPECIAL MAINTENANCE STEERS; IN PER CENT. OF FRESH WEIGHT.

No. of steer.....	593	597	595	591	592
Head and tail.....	4.59	4.63	4.99	4.48	4.42
Feet.....	4.59	4.38	4.72	3.89	4.34
Shins and shanks....	4.44	5.21	5.56	4.54	4.90
Chucks and neck....	4.89	4.71	5.18	4.75	3.99
Flanks and plates....	3.12	3.09	3.63	3.35	3.17
Ribs.....	5.06	4.47	5.22	4.75	4.11
Loins.....	4.61	4.84	5.18	4.25	3.82
Rumps.....	4.32	4.55	4.49	4.68	4.34
Rounds.....	5.28	4.49	4.83	4.45	4.05
Total skeleton.....	4.40	4.61	4.99	4.41	4.16

Although steer 592 was the second oldest of the six, the ash content of his skeleton was lowest, in the skeleton as a whole. This may be a mere coincidence or it may point to a resorption of mineral matter from the bones during a long period of insufficient nutrition. No conclusion can be

drawn from only one animal. Compare the recent work of Hart¹ and others at the Wisconsin Agricultural Experiment Station.

Phosphorus.

The percentages of phosphorus, reported in Tables I and VII, are those obtained by determination of the phosphorus in the ash.

In order to show that very little phosphorus is dissolved by the ether, determinations of phosphorus were made upon each sample of fat extracted from the skeletons of steers 591, 593 and 597. The results show that the amount of phosphorus lost in this way is entirely negligible. The amount of phosphorus in the fat was less than 0.06 per cent. in all cases, averaging about 0.02 per cent. of the weight of the fat, or calculated to the fresh skeleton was less than 0.003 per cent.

The phosphorus content reckoned upon the original substance gives about the same indication as does the ash. Those steers having the highest total moisture and fat content, or, in other words, those having the least solid matter are lowest in phosphorus. Calculated to the dry and fat-free basis, the phosphorus content also corresponds to that of the ash.

The composition of the ash, as indicated by the per cent. of phosphorus, varies in the different parts of the skeleton. The per cent. of phosphorus in the ash of the single cuts varies differently in the several steers and no conclusion will be attempted from the present data.

CONCLUSIONS.

(1) Young, growing steers continue to grow in height and build up skeleton even when losing in weight.

(2) The skeleton is unaffected by poor nutrition until practically all of the fat has been removed from the muscles and other organs.

(3) The principal effect of poor nutrition upon the skeleton is the removal of the fat or marrow and the replacement of this with water.

(4) The per cent. of organic matter other than fat is practically constant for the whole skeleton, under different conditions of nourishment.

(5) No evidence was obtained to warrant the conclusion that the mineral matter is resorbed or affected in amount due to lack of proper nourishment, although in steer 592 there is some indication that this may have taken place.

¹ Hart, McCullum and Fuller, *Am. Jour. Physiol.*, **23**, 246 (1909).
Hart, McCullum and Humphrey, *Ibid.*, **23**, 86 (1909).

(6) The proportion of fat and moisture in the corresponding parts of the skeleton is fairly constant for normally fed steers. In steers which have suffered from insufficient nutrition for a long period the fat may be nearly all resorbed from the skeleton, and this resorption takes place from all parts of the skeleton.

(7) The proportion of organic and mineral matter in the skeleton varies with the age. This proportion varies in the different parts of the skeleton, according to the nature of the bone.

(8) The per cent. of phosphorus in the ash of the skeleton of steers is nearly constant. The per cent. of phosphorus in the ash of different parts of the skeleton of the same steer varies, but the average for the corresponding cuts of the five steers is fairly constant, showing a variation of not more than 0.7 per cent.

UNIVERSITY OF MISSOURI,
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EMIL CHR. HANSEN.

MAY 8, 1842—AUGUST 27, 1909.

Last year, on the 12th of November, an anniversary of no little importance was celebrated by the Carlsberg breweries in Copenhagen. On that day it was 25 years since the first pure culture yeast was introduced in the brewery of "Gamle Carlsberg." And to Emil Chr. Hansen justly went all the honors of the day.

Before Hansen made his epoch-making discoveries the brewing industry had been in the dark in regard to the causes of the very disturbing and often fatal troubles which occurred in the beer during fermentation and storage. Pasteur ascribed the diseases to the influence of bacteria and prescribed methods for the purification of the yeast, methods which in many cases not alone did not improve the conditions but even made them worse. Pasteur's methods were to cultivate the yeast in a sugar solution to which had been added tartaric acid or in beer-wort containing a small percentage of carbolic acid in order to destroy the bacteria. In many, and perhaps the most cases, however, the diseases of the beer were not due to bacteria but, as Hansen showed, to certain species of yeast, wild yeasts, and as the addition of tartaric acid and carbolic acid to the fermenting liquid did more damage to the good yeast than to the wild yeast, the result was the opposite of what was expected. The only solution which might

prove satisfactory seemed to be the elimination of the wild yeast as well as the bacteria. But how? This Hansen accomplished by introducing his pure culture method. Hansen devised methods by which it was possible to distinguish between the different species of wild and culture yeast. Hansen, and not Robert Koch, was the first to introduce nutrient, transparent gelatine as a highly suitable medium in which to grow cultures. Hansen proved that by using pure cultures in the fermentation of the beer-wort, it was always possible to obtain the same result. If the starting yeast was kept pure and precautions were taken that no infection was introduced during the different stages of manufacture, the brewer would always be sure that he would obtain the same good and stable product. In connection with Kühle, the late director of "Gamle Carlsberg," he constructed a pure culture apparatus by means of which it was possible to work the pure cultures on a scale large enough for practical purposes.

From the time the pure culture yeast was first introduced in "Gamle Carlsberg" brewery, it has been a great success. With the generosity which always has characterized the Jacobsens (J. C. Jacobsen the founder of "Gamle Carlsberg," and his son, Carl Jacobsen), the discoveries of Hansen were given to the world, and every brewery in the old as well as in the new world has benefitted more from these than from any other discovery of modern times.

Not the breweries alone, but also the other branches of the fermentation industry: the distilleries, the compressed yeast factories, the wine factories have benefitted more or less from these same discoveries of Hansen. Also other industries have taken advantage of the pure cultures. Thus it was due to the introduction of the pure culture of lactic acid bacteria in the dairies that the Danish butter has reached the highest grade of perfection and won the reputation of being the best in the world.

Hansen limited his research work to the yeasts and to the bacteria which occur in the fermentation of beer, but these fungi he also pursued wherever and whenever he found them, not alone in the beer but also in the air, in the water, in the earth until they revealed their last life-secret to him. It is a fact that his results are final, at least, nobody has ever yet succeeded in disproving any of his printed statements.

Emil Chr. Hansen came from a poor family. His highly eccentric father was a painter by trade,