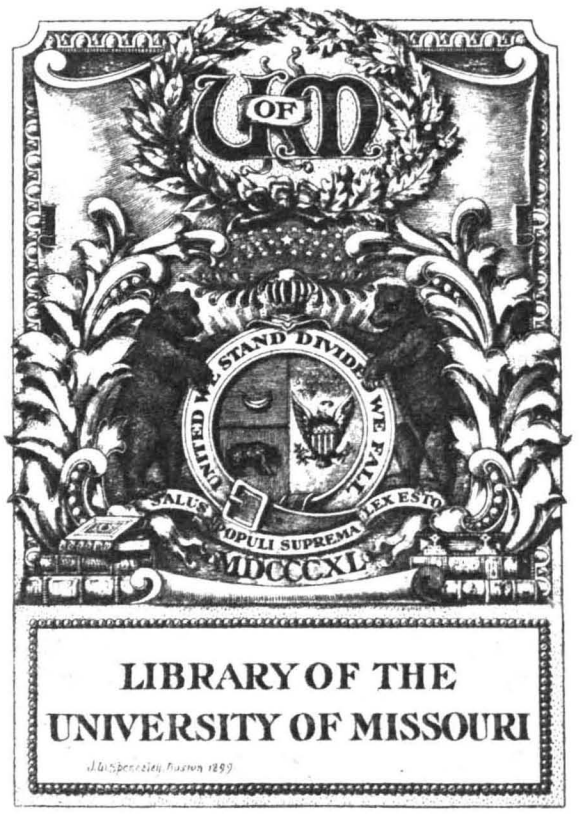

AN INVESTIGATION UPON THE NITROGEN, PHOSPHORUS, SULFUR, AND ASH CONTENT OF THREE BEEF ANIMALS, WITH A SPECIAL STUDY UPON THE FORMS OF SULFUR

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Part I

Part II

Part I

NITROGEN, PHOSPHORUS, SULFUR, and ASH DATA

Part I

Historical

The work of Lawes and Gilbert[1] of the Rothamsted Experiment Station upon the composition of animals fed and slaughtered as human food is the first work published on the composition of beef animals. These classical experiments started in April, 1848, the animals being slaughtered at intervals until December, 1853. The first published work appeared in June, 1858, and contains the results of the analysis of one calf, two oxen, one lamb, four sheep, and two pigs.

A supplementary paper[2] to the above publication appeared in June, 1883, and dealt wholly with the composition of the mineral matter of entire animals analyzed, and of the separated parts.

The authors point out many interesting points shown by these data. Taking up first their table of the mineral matter, we see that the fresh carcass contains from four to seven times as much mineral matter in the bones as in the total flesh. The per cent of mineral matter in the soft parts is in all cases greater for the animal in the lean condition than for that in the fat condition. In the case of beef, the per cent of mineral matter in the soft parts of the offal averages slightly lower than that in the soft parts of the carcass. Considering a composite sample of the carcass we find an average of 4.869 per cent ash, the soft parts furnishing 0.729 per cent ash, and the bones fur-

nishing 4.14 per cent of ash. As the animal fattens the per cent of mineral matter referred to entire animal decreases in both bones and soft parts, showing that its increase is in a smaller proportion to the other constituents during the fattening period.

In the nitrogen tables it is seen that in the fresh carcass in the case of beef there is an average of 4.6 times as much nitrogen in the soft parts as in the bones. If no use is made of the carcass bones as food, about one-fifth of the carcass nitrogen is lost for this purpose. As the animal fattens the per cent of nitrogen in the carcass decreases. The authors state: "It will be seen further on, that the fattening or maturing is accompanied by a considerable diminution in the percentage of water in the body. The dry matter accumulated consists, however, in a much greater proportion of fatty substance, than of nitrogenous compounds. Indeed it would seem probable that, necessarily, the larger the amount of the nitrogenous compounds, the larger the amount of water required for their proper hydration, for the purpose they subserve in the system."

The authors have compared the determinations of nitrogen obtained in three different ways. From the percentage of crude dry substance was subtracted the per cent of mineral matter and fat found by analysis, the result being the

per cent of nitrogenous material. The per cent of nitrogen found by dividing by the factor 6.3 was compared to the per cent nitrogen found by a determination upon the composite crude dry substance, and also to that found by adding together amounts of nitrogen found in each separate crude dry substance. The results by these three methods compare very favorably with each other, altho the results obtained by direct analysis are all higher than those obtained by calculation. Had the factor 6.25 been used instead of 6.3, an average of the ten animals would show only 0.014 per cent lower nitrogen content than the results obtained by addition of the per cent of nitrogen found in the various organs. In the offal parts of the beef animals (excluding contents of stomachs and intestines) the nitrogen averages 0.456 per cent higher than that in the fresh carcass. The table giving the summary of the mean percentages of nitrogen shows 66.6 per cent of the total nitrogen of the fasted live weight of beef animals to be in the carcass parts. About one-fifth of this is in the carcass bones, so the soft and edible portions of the carcass will contain about one-half of the entire nitrogen of the body. Since some parts of the offal are edible, a part of the 33.3 per cent remaining may be utilized as food. The results on water, nitrogenous material, fat, and mineral matter, show a tendency of the per cent of nitrogenous compounds to rise and fall with the per cent of mineral matter. This seems to indicate some relation between the material furnishing the

mineral constituents and the nitrogen-containing materials in the body.

Again the authors state, ''Whilst the percentages of both mineral matter and nitrogenous substance decrease, as the animals mature, that of the fat, on the other hand, very considerably increases. Indeed, the increase in the percentage of fat is much more than equivalent to the collective decrease in that of other solid matters: that is to say, as the animal matures, the percentage in its carcass, of total dry substance (and especially of fat) much increases. There is then, of course, a corresponding diminution in the proportion of the water.'' This is shown by the percentage of water in the carcasses of the three animals analyzed: calf, 62.25 per cent; half fat ox, 54 per cent; fat ox, 45.5 per cent.

The proportion of bone becomes smaller as the animal becomes fatter, but the percentage of dry matter in the bone increases as the animal matures.

Composition of the ash of certain separated parts of the entire animals slaughtered for human food

This is supplementary to the first report of the authors[2] and was published in June, 1883. They report the results of the analysis of ash from the carcasses, from the offal parts, and from the entire animals. The ashes were obtained by burning the composited crude dry substance of the parts making up these divisions. Twelve analyses were made upon

ashes of carcass, seventeen analyses upon ashes of offal parts, and eleven analyses upon ashes of entire animals.

For the ashes of the entire animal the results are reported for crude ash and pure ash. The latter report is the same as the former, excluding the sand and charcoal. Following is the report of the crude and pure ash of the three animals, figured upon their fasted and live weights as 100. The contents of the stomach and intestines are not included in the parts analyzed.

Percentage of Crude Ash and of Pure Ash in the Fasted Live Weight

Composite	CRUDE ASH			PURE ASH		
	Carcass Parts o/o	Offal Parts o/o	Entire Animal o/o	Carcass Parts o/o	Offal Parts o/o	Entire Animal o/o
Fat Calf	2.782	1.018	3.800	2.772	1.006	3.779
Half Fat Ox	3.603	1.061	4.664	3.568	1.044	4.612
Fat Ox	3.019	0.901	3.920	2.997	0.882	3.879

It may be said in general in regard to the ash that the animal ash consists largely of tricalcium phosphate. The authors again state, ''But it may nevertheless be useful to indicate the general relation of base to acid in the ashes. The lime and magnesia may be taken as essentially, tho of

course not exclusively, representing the bases of the bone ash; while the potash and soda may in the same general, tho not in an exclusive, sense be classified as the flesh and blood bases. Again, by far the larger proportion of the phosphoric acid will be due to the bones; whilst some of it as such, and probably some as the product of the oxidation of phosphorus in the burning, will be connected with the nitrogenous constituents in the non-bony portions of the body.''

Some meat investigation work was carried on by Schweitzer[3] of the Missouri Agricultural Experiment Station. These investigated samples were not prepared from entire organs but from specially selected cuts and do not offer any special comparative data for this discussion.

Emmett and Grindley[4] have reported analyses of beef kept in cold storage and in the fresh condition. Two steers were slaughtered, the carcasses divided into halves as usual and placed in cold storage. The round, rib, plate, and loin were taken for analysis from one half of the carcass of one animal after two days, and the same cuts from the other half after a further interval of twenty-two days. From the other animal the chuck and loin from one half of the carcass were taken from storage for analysis after six days, and the same cuts from the other half of the animal after being thirty-seven days longer in storage. Some of the conclusions of the authors are: no apparent change

in the forms of ash, protein, and solids, and only a small change in the soluble phosphorus and non-nitrogenous extractives, and the nutritive value unaltered, after 22 days; after 37 days the change of the forms of solids, protein, phosphorus, and extractives was noticeable, but as far as nutritive value was concerned, no appreciable amount was lost; on the contrary there was a gain rather than otherwise, due to an increase in soluble proteins and extractives.

Trowbridge and Woodman[5] offer the following conclusions with their work on five steers:

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

2. The skeleton is unaffected 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, in consequence of lack of proper nourishment, altho in one steer there is some indication that this may have taken place.

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 age. This proportion also 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 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.''

The results of malnutrition upon the water and fat content of the body of animals have been pointed out by Voit[6], who states that in the case of poor nourishment the whole body becomes watery. A well nourished organism, on the contrary, contains more dry substance, since in it there is more fatty tissue with less water content.

Francis and Trowbridge[7] show that phosphorus in beef animals gives the following results:

1. The offal systems contain the highest percentages of phosphorus of all the parts of the animal (not

including the bone), the subsequent order being: nervous system, liver, respiratory system, digestive and excretory system, circulatory system.

2. The fat carcass cuts, or in the thin animals the cuts containing most connective tissue, are lowest in phosphorus; the cuts high in lean meat are relatively high in phosphorus.

3. In a moisture and fat free condition these cuts (lean) which are highest in connective tissue are lowest in phosphorus.

4. There seems to be no relation between ash and phosphorus content.

Analytical and feeding experiments are reported by Heubner[8], who studied the phosphorus of the bodies of laboratory animals (dogs). In young animals of from 2 to 7 pounds weight the phosphorus of the organism showed very little variation and averaged 0.6 per cent of the total body weight. Of the phosphorus content, 0.15 per cent was found in the muscle, 0.2 per cent in the central nervous system, liver and kidneys, and most of the remainder in the bones. Lack of phosphorus in the diet decreased the per cent of phosphorus in the body only when growth was indifferent. Under this condition the greatest loss occurred in the bones.

''Investigations made on calves and sheep indicate that the proportion of bone to the total weight of the

animal varies inversely with the age, the quality and the weight of the animal. The proportion of bone is found to be very variable and the weight of bone reaches and sometimes exceeds one-third of the total weight of the animal.[9]"

According to Plimmer[10] "The first work which shows the presence of sulfur in protein was done by Mulder and was followed by investigations by Fleitman in Liebig's laboratory in 1847". These results while most interesting do not offer data for more than very general comparisons. There has been very little work done on the sulfur content of beef animals. Most of the results reported are sulfur determinations upon ashed materials, which results have been shown by Berthelot[11], Barlow[12], Fraps[13], Goss[14], Beistle[15], Sherman[16], and others to be low and not a true measure of the sulfur content of the original material. The losses of sulfur on ashing are not constant but vary within wide limits without any degree of regularity, making comparisons with total sulfur determinations entirely out of the question. There has been a large amount of work done on sulfur metabolism, particularly with reference to nitrogen.

The discovery of sulfur in cow's milk was reported by Sartori[17] in 1893 to be 0.043 per cent, and Kojo[18] has reported the sulfur content of human blood to be 0.2 per cent. Human hair has been reported by Rutherford and Hawk[19] to

contain 4.95 per cent sulfur, while Mulder[20] reports as high as 2.8 per cent for nails.

In unpublished data from this Station[21] the sulfur content of a newborn calf is reported 0.212 per cent sulfur on the fresh basis which is 0.911 per cent when reduced to the moisture and fat free basis. Katz[22] reports the per cent of sulfur of the dry muscle of various animals as shown in the following table:

Animal	Per Cent Sulfur Dry Basis
Man	0.7576
Pig	0.7536
Ox	0.7719
Calf	0.9178
Deer	0.8517
Rabbit	0.8500
Dog	0.9643
Cat	0.8748
Hen	0.9234
Frog	0.8835
Haddock	1.1514
Eel	0.3657
Pike	1.0576

Part II

Experimental

The animals used in this study were typical steers of the Southwest. The following table shows details in regard to dates of birth, slaughter, and respective weights.

Animal No.	Date of Birth	Breed	Date of Slaughter	Group	Age at Slaughter	Weight at Slaughter lbs.
548	4/21/12	Registered Shorthorn	9/30/12	III	5 Mo.9 Da.	218.82
552	4/23/12	Hereford-Shorthorn	9/30/12	II	5 Mo.7 Da.	256.82
557	4/13/12	Grade Shorthorn	9/30/12	I	5Mo.17 Da.	451.81

These animals were carefully selected, were very uniform and of a good feeding type and were weaned and placed under experiment within three weeks of date of birth.

The animals were started on a milk diet and were offered hay at the age of about three weeks, and grain one week later. Group I was full fed and crowded, Group II was fed for maximum growth without laying on appreciable fat, Group III was fed for retarded growth. Collectively they were known as the ''Use of Food'' group. The variable factors in this experiment were age and quantity of feed; the invariable factors were quality of feed, breed, unsexed animal, and seasonal influences.

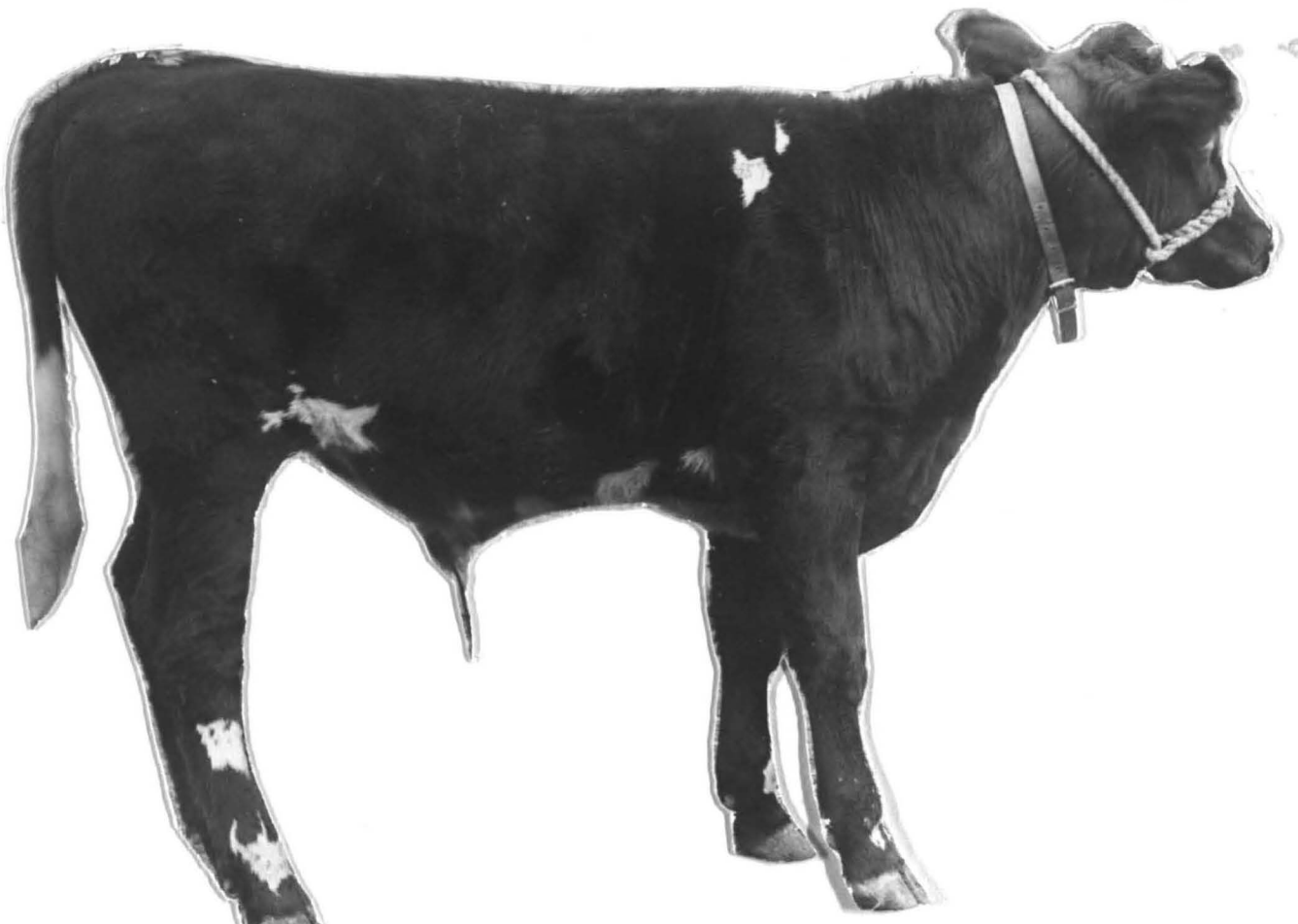
At the age of practically five months the animals were

PHOTOGRAPH OF ANIMAL 548 TAKEN TWO DAYS

BEFORE SLAUGHTERING

PHOTOGRAPH OF ANIMAL 552 TAKEN TWO DAYS BEFORE SLAUGHTERING

PHOTOGRAPH OF ANIMAL 557 TAKEN TWO DAYS BEFORE SLAUGHTERING



slaughtered, being stunned, hoisted by the hind feet and thoroly bled into tared containers. The bleeding was assisted by pumping the forelegs. The main portion of the blood was weighed and immediately the volume was measured. Blood dripping from the carcass was caught and weighed later. Weights of all parts, organs, and cuts of meat were taken.

Much of this discussion will concern itself with composite samples taken from these three animals, and their comparison to the composite samples of other animals taken in a similar manner. The carcasses were divided as nearly as possible into halves and the composite samples taken from the right half of each carcass.

The composites were as follows:

1. Hair and hide of entire right half of carcass.
2. Horn and hoof and dew claws of entire right half of carcass.
3. Blood of entire animal.
4. Lean and fat of entire right half of carcass, including lean and fat of right half of head and tail.
5. Skeleton of right half of entire animal.
6. Internal composite, including offal fat, circulatory, respiratory, and nervous systems, and digestive and excretory system.

These different samples of internal organs and wholesale cuts were analyzed directly as fresh material for mois-

ture, fat, ash, nitrogen, and phosphorus. Large composites were preserved for a future study of the sulfur content. The sulfur was analyzed from these composite samples. From the analyses of the parts making up the composite samples used for sulfur investigation, the nitrogen, phosphorus, sulfur, and ash were all brought to the same basis for comparison.

In order to preserve these composite samples, special care was taken in regard to the size of sample and amount and condition of the alcohol. The composite samples were preserved with alcohol which had been freed from acetic acid by distillation over sodium hydroxide. The size of the samples preserved varied from 500 to 1500 grams. The moisture content of the fresh sample was taken into consideration and sufficient alcohol was added to fill the jar and retain the concentration of the alcohol above 50 per cent. Of the eighteen composite samples the jar containing the internal composite of 557 was broken and in one sample (blood, 552)* putrefaction had set in. This sample was, however, analyzed and its condition carefully noted to ascertain whether or not there was a loss of sulfur.

The preserving jars having been opened, the contents were carefully washed into large evaporating dishes with alcohol and evaporated to dryness, additional alcohol being added from time to time to facilitate the driving off of water. The blood, hair and hide, and horn and hoof sam-

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*Possibly the container was not air tight and there was undue evaporation of alcohol.

ples were brought to an air dry condition and dried without removal of fat. The skeleton, lean and fat, and internal composites required removal of excess fat by ether before grinding could be carried out. This was done by putting the samples into large conical funnels containing a perforated plate in the bottom, two and one-half inches in diameter. On top of this plate a sheet of filter paper was placed to prevent the loss of small particles of the sample. The samples were extracted with cold ether until sufficient fat was removed to permit further grinding of the samples. After being washed with ether the samples were spread out upon a paper and freed from ether by a gentle blast of air from an electric fan, and were then brought into uniform condition by passing thru a drug mill.

Moisture determinations were made by drying by vacuum without heat[23]. The fat determinations were made by the regular Soxhlet extractor method. For nitrogen, the modified Kjeldahl-Gunning method was used. The phosphorus and ash were determined as outlined in the Bureau of Chemistry Bulletin 107 (Revised).

Methods for Determination of Total Sulfur

The literature covering the methods for the determination of total sulfur was carefully reviewed in order to obtain the most reliable method of procedure. The methods can be grouped as follows: (1) oxidation of the substance by means of nitric acid in a closed tube, (2) heating in a com-

bustion tube in the presence of a current of oxygen, (3) fusion with an alkali and oxidation with an oxidizing agent or treatment of the substance in an alkaline solution with chlorine or bromine.

Beistle[15] tested out eight different methods and reported most favorably for the fusion with potassium hydroxide and potassium nitrate.

Sherman's[16] results on three different methods are shown in the following table.

	Compressed Oxygen o/o	Hydrogen Peroxide o/o	Nitric Acid Method o/o
Dried Lean Beef	0.815	0.811	0.679
Dried Curd	0.710	0.608	0.495
Yolk of Egg	0.822	0.819	0.810
White of Egg	1.408	1.421	1.146
Wheat Bran	0.264	0.259	0.182
Beans	0.217	0.229	0.165
Average	0.689	0.691	0.580

Sherman says: ''While the results obtained by the first two methods are equally accurate, the combustion in oxygen has some distinct advantages.''

It is not the purpose of the writer to bring in a long discussion of the merits of these methods, but it seems desirable to state that the peroxide fusion or Osborne[24] method is looked to as a standard of comparison in sulfur

determinations, and while the compressed oxygen method appears to be equally accurate, it was not as feasible as the peroxide method in this case. The compressed oxygen method was first described by Berthelot[25] in 1892 and later modified by Hempel[26]. As employed by Sherman[16] it is essentially the Hempel method with slight changes in technique. The nitric acid method referred to in the table was carried out as described by Fraps[27] in 1902.

The sulfur determinations of the present investigation were made by the Osborne[24] method. It was necessary to make variations in the technique to obtain best results.

Ten grams of sodium peroxide were placed in a nickel crucible and just enough water was added to complete all reaction. The crucible was next heated until nearly all of the water was expelled; this was indicated by the formation of a scum on removing from the flame and cooling slightly. Two grams of the air dried sample was added and thoroly mixed with the contents of the crucible with a platinum stirring rod. The crucible was placed over an alcohol flame (any flame free from sulfur might be used) and the heating continued slowly with constant stirring of the contents of the crucible until all frothing had ceased and the mass had subsided. The color of the contents of the crucible changed suddenly from a brown to a black substance having an oily appearance. It was at this stage of the fusion that greatest care was needed. With samples

containing much fat (such as the hair and hide not extracted with ether) it was necessary to heat very cautiously until this stage was passed in order to avoid flashing of the contents of the crucible. After heating for several minutes a piece of sodium peroxide about the size of a pin head was dropped into the crucible. If a snapping or flashing was observed, the liquid mass was heated further without adding more sodium peroxide. Small quantitites of the peroxide were added until the oxidation was complete. The fused mass was cooled and dissolved in water, transferred to a beaker, strongly acidified with HCl and boiled to drive off the excess of chlorine. The solution was filtered hot and washed until the filtrate was free from chlorine. The filtrate and washings were exactly neutralized with ammonium hydroxide and 4 c.c. of concentrated HCl was added. If necessary the solution was evaporated to 400 c.c. volume. Ten per cent hot barium chloride solution was added to the boiling solutions. The precipitating solutions were boiled one-half hour and allowed to stand on a warm steam bath over night. The precipitate was washed with a solution containing a little HCl and then with water until free from chlorides. It was then dried, ignited, and weighed. The filtrations were all made thru blue ribbon 589 S. and S. filters.

Discussion of Nitrogen Data

The following table shows the per cent of nitrogen in

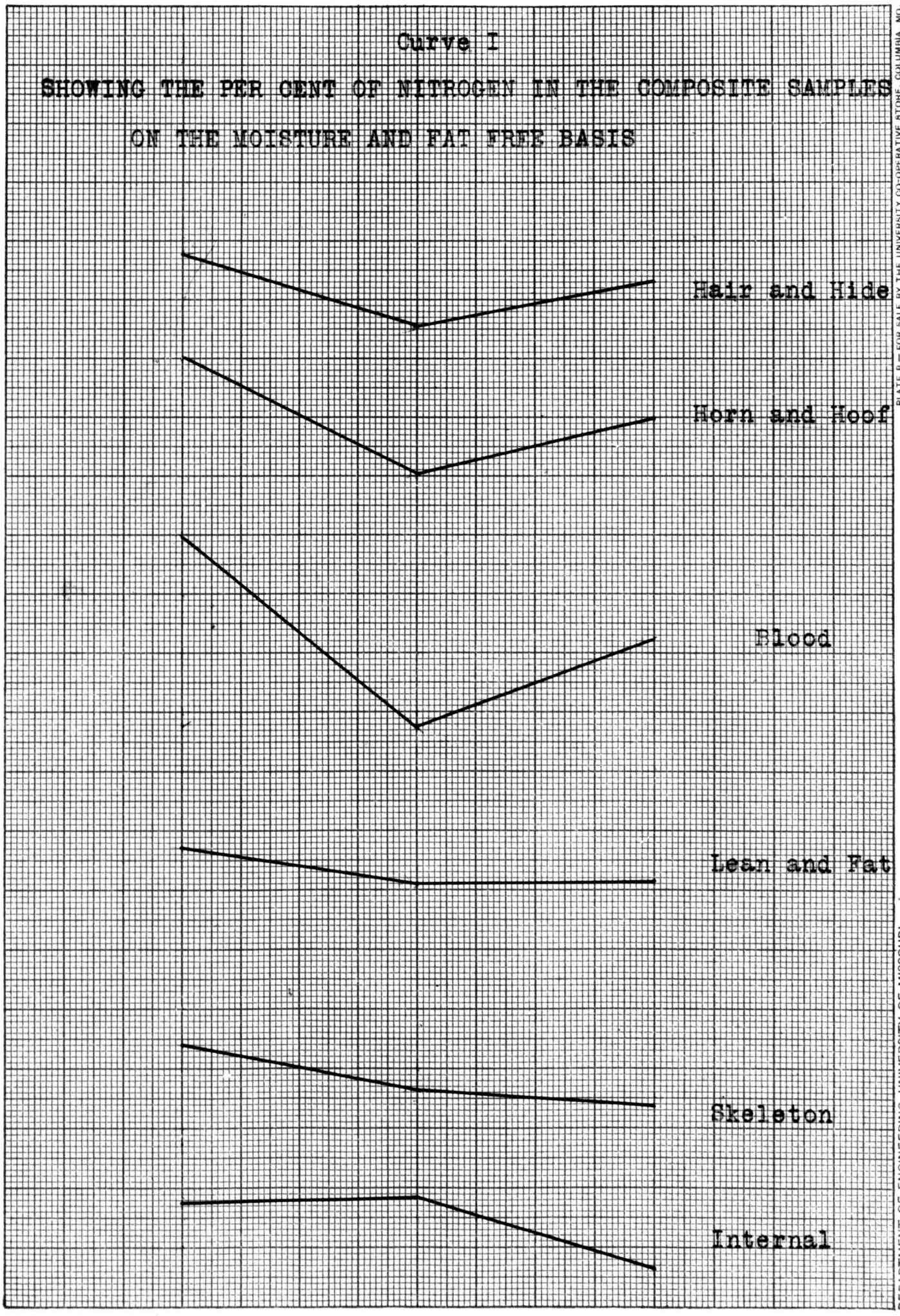
the various composites. The hair and hide, horn and hoof, blood, and lean and fat composites, all range between 14.5 and 16.5 per cent of nitrogen, while the internal composite drops to an average of 13.72 per cent. The skeleton composite sinks to an average of 7.91 per cent. The per cent of nitrogen in the blood of 548 is the highest of any of the composites, while the skeleton composite of 557 shows the lowest. In Group III the hair and hide, and horn and hoof show a higher per cent of nitrogen than either Group II or Group I, Group II being the lowest. The nitrogen content of the blood for Group II is lowest, increasing in both Group I and Group III. The lean and fat remains a fairly constant per cent for the three animals, Group III being slightly higher than Groups II or I. The skeleton of Group III shows the highest per cent of nitrogen, Group II being lower, and Group I the lowest. This seems to indicate that as the animal gains in flesh and therefore in weight there results a heavier bone having a larger per cent of mineral matter and a smaller per cent of nitrogen.

If we turn to the nitrogen curve No. 1 we find that the Group III animal shows for every composite the highest nitrogen content with the exception of the internal composite which is 0.07 per cent higher in the case of the Group II animal than in the case of the same composite for Group III. Group I has a higher nitrogen content than Group II, excepting skeleton and internal composite samples which are 0.164 per cent

Curve I

SHOWING THE PER CENT OF NITROGEN IN THE COMPOSITE SAMPLES

ON THE MOISTURE AND FAT FREE BASIS



548 III

552 II

557 I

and 0.619 per cent lower respectively. Taking the composite of the entire animal, Group III has 12.695 per cent, Group II, 12.301 per cent, and Group I, 12.411 per cent. This shows Group II, the animal which has the optimum conditions for growth, to contain the smallest per cent of nitrogen. According to Voit[6] there is more watery tissue in a poorly nourished animal, and according to unpublished data of the Missouri Station there is a very strong indication of the replacement of fat by water.

Table I

NITROGEN IN COMPOSITE SAMPLES ON MOISTURE AND FAT FREE BASIS

Composite Samples	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Hair and Hide	15.883	15.258	15.645
Horn and Hoof	15.988	15.005	15.485
Blood	16.487	14.882	15.635
Lean and Fat	14.855	14.556	14.565
Skeleton	8.227	7.828	7.664
Internal	13.879	13.949	13.330
Composite of Total Animal	12.695	12.301	12.411

Table II

WEIGHT OF COMPOSITE SAMPLES ON THE MOISTURE AND FAT FREE BASIS AND WEIGHT OF NITROGEN IN THESE COMPOSITE SAMPLES

	Steer 548, Group III		Steer 552, Group II		Steer 557, Group I	
Composite Samples	Weight of Composite Grams	Weight of Nitrogen Grams	Weight of Composite Grams	Weight of Nitrogen Grams	Weight of Composite Grams	Weight of Nitrogen Grams
Hair and Hide	2813.22	446.82	3332.64	508.48	4492.83	703.31
Horn and Hoof	213.28	34.10	254.86	38.24	349.46	54.11
Blood	865.46	142.69	1055.59	157.09	1795.50	280.73
Lean and Fat	8532.50	1267.52	9813.73	1428.48	15525.43	2261.29
Skeleton	7520.95	618.76	8590.26	672.42	12223.93	936.40
Internal	1864.94	258.84	1908.91	266.29	3534.27	471.10
Total Nitrogen		2768.73		3071.00		4706.94

Table III

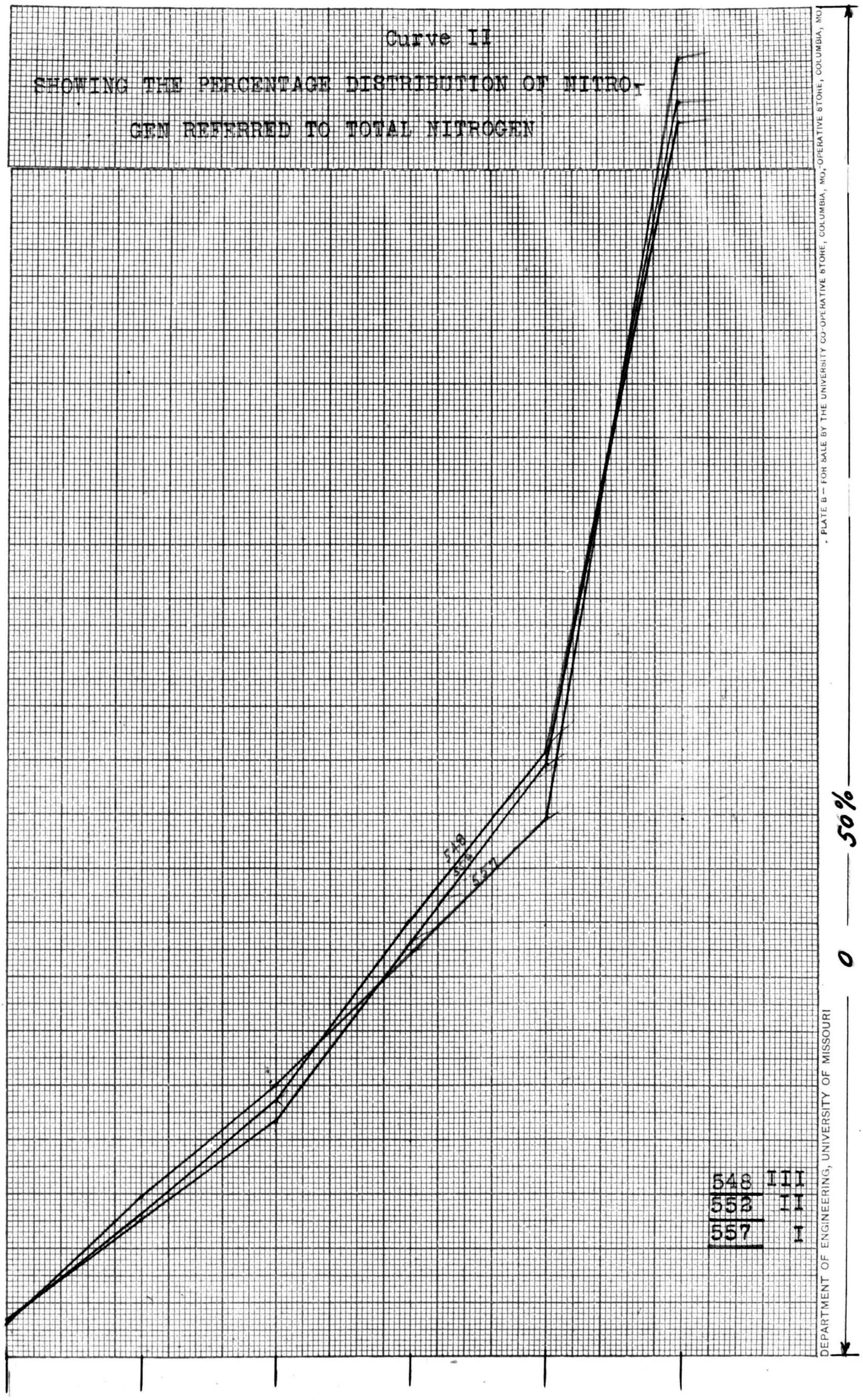
PERCENTAGE DISTRIBUTION OF NITROGEN REFERRED TO TOTAL NITROGEN

Composite Samples	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Lean and Fat	45.78	46.52	48.04
Skeleton	22.35	21.90	19.90
Hair and Hide	16.14	16.56	14.94
Internal	9.35	8.67	10.01
Blood	5.15	5.11	5.96
Horn and Hoof	1.23	1.24	1.15
	100.00	100.00	100.00

Table II shows 2768.73 grams, 3071.00 grams, and 4706.94 grams of nitrogen in animals 548, 552, and 557 respectively. Does the plane of nutrition affect the relative deistribution of the nitrogen? This is clearly shown in Table III and in Curve 2. We note the distribution of nitrogen in the three animals referred to total nitrogen is rather variable. The slow growth animal shows relatively less nitrogen in the lean and fat composite and a greater relative amount of skeletal nitrogen than does the Group I animal. The Group I animal shows a smaller relative amount of nitrogen in the hair and hide, which may be due to a greater relative increase in the lean and fat

Curve II

SHOWING THE PERCENTAGE DISTRIBUTION OF NITROGEN REFERRED TO TOTAL NITROGEN



tissue than in the hair, which is somewhat higher in nitrogen. There is 2.2 per cent more nitrogen in the lean of 557 and 2.3 per cent less nitrogen in the skeleton.

The per cent of nitrogen in the blood referred to total nitrogen is greatest in the Group I animal, indicating a greater proportionate amount of blood in Group I than in the other two groups.

Discussion of Phosphorus Data

Table IV and Curve 3 showing the per cent of phosphorus show the highest per cent and the widest variation in the per cent of phosphorus to be in the skeleton. The smallest per cent of phosphorus, 0.221 per cent, is found in the blood, increasing in the hair and hide, horn and hoof, lean and fat, and internal composite in the order named, until the skeleton shows in one case 8.43 per cent.

The composites of the total animals show 3.284 per cent, 3.373 per cent, and 3.192 per cent phosphorus for Groups III, II, and I, respectively. The distribution of the phosphorus in each of the entire animals is shown in Table V, there being a total of 716.3 grams, 841.49 grams, and 1210.41 grams for 548, 552, and 557 respectively.

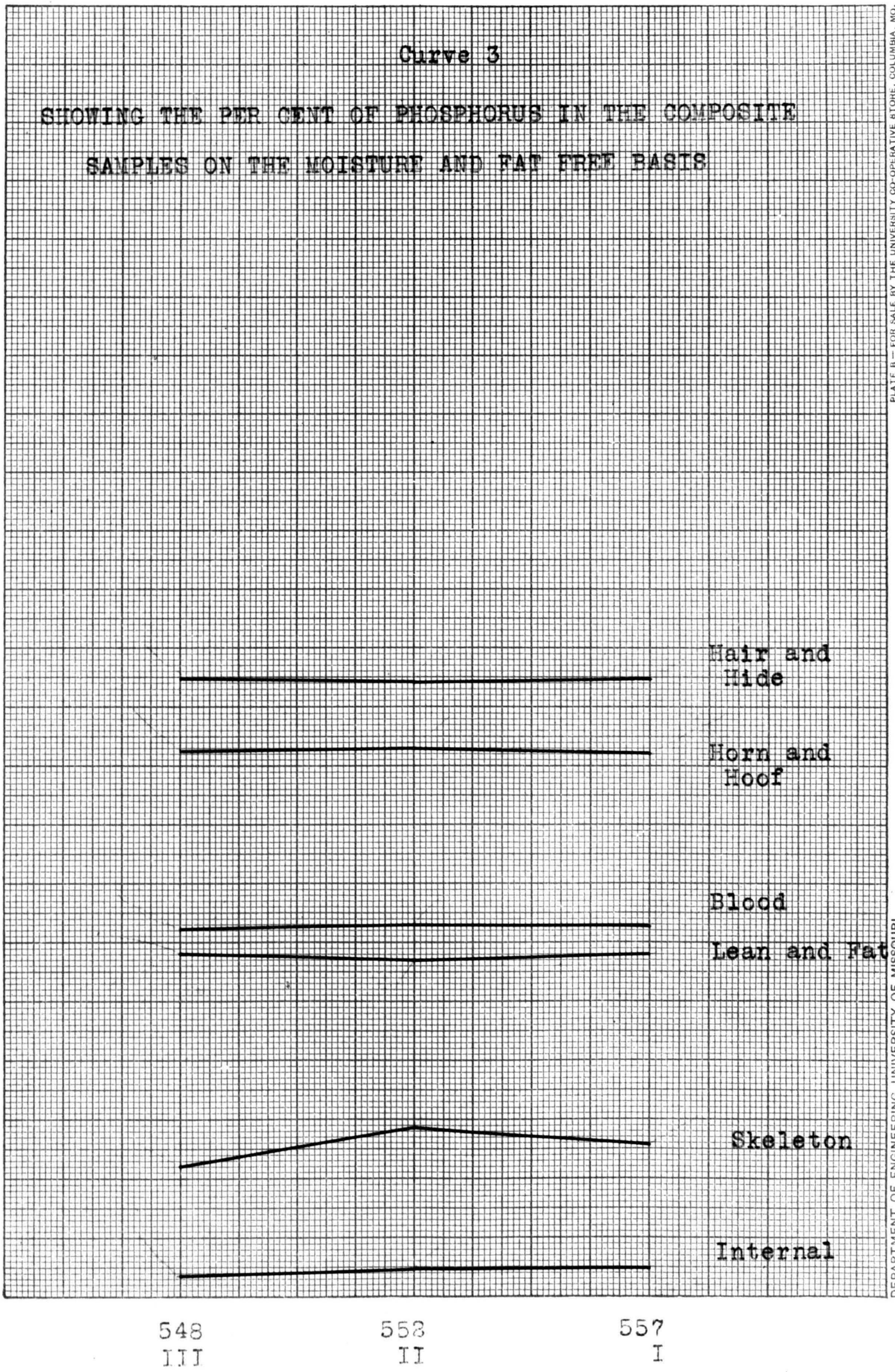
Table IV

PHOSPHORUS IN COMPOSITE SAMPLES ON MOISTURE AND FAT FREE BASIS

Composite Samples	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Hair and Hide	0.232	0.221	0.222
Horn and Hoof	0.610	0.643	0.621
Blood	0.112	0.138	0.135
Lean and Fat	0.891	0.853	0.906
Skeleton	8.104	8.430	8.277
Internal	1.177	1.224	1.228
Composite of Total Animal	3.284	3.372	3.192

Curve 3

SHOWING THE PER CENT OF PHOSPHORUS IN THE COMPOSITE SAMPLES ON THE MOISTURE AND FAT FREE BASIS



±1-2% ± 8-9% ± 0-1% ± 0-1% ± 0-1% ± 0-1%

Table V

WEIGHT OF COMPOSITE SAMPLES ON THE MOISTURE AND FAT FREE BASIS AND WEIGHT OF PHOSPHORUS IN THESE COMPOSITE SAMPLES

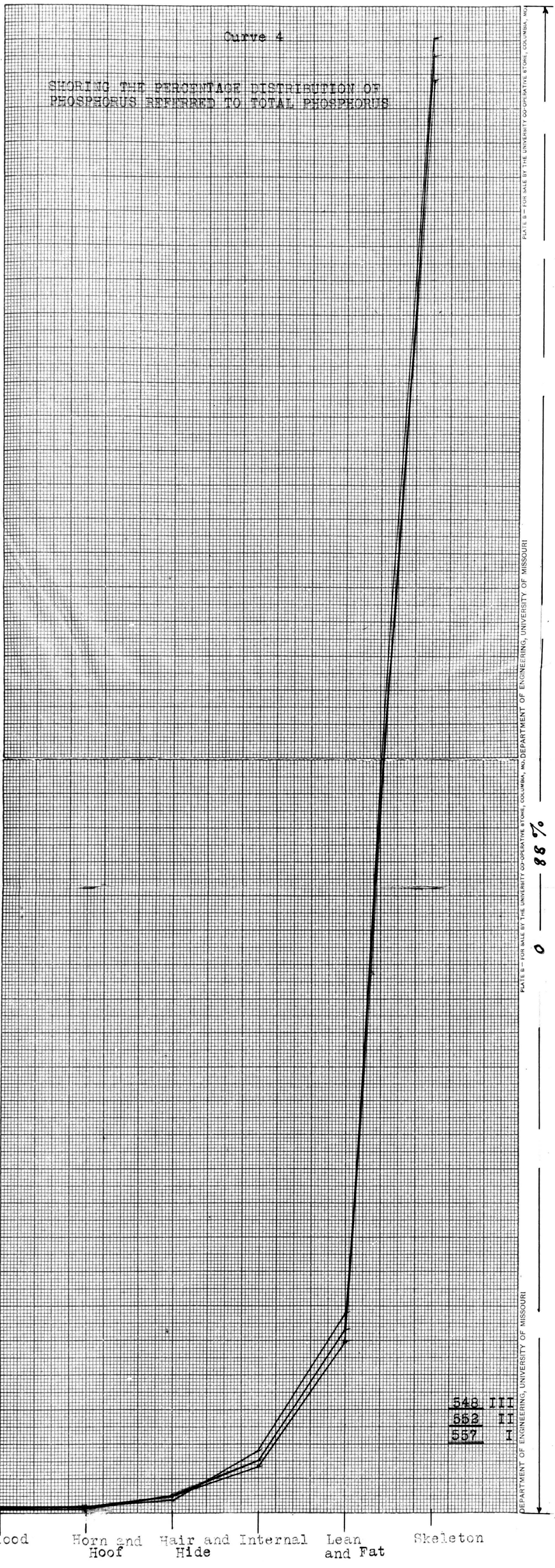
	Steer 548, Group III		Steer 552, Group II		Steer 557, Group I	
Composite Samples	Weight of Composite Grams	Weight of Phosphorus Grams	Weight of Composite Grams	Weight of Phosphorus Grams	Weight of Composite Grams	Weight of Phosphorus Grams
Hair and Hide	2813.22	6.52	3332.64	7.37	4492.83	10.01
Horn and Hoof	213.28	1.30	254.86	1.64	349.46	2.17
Blood	865.46	0.97	1055.59	1.46	1795.50	2.42
Lean and Fat	8532.50	76.06	9813.73	83.70	15525.43	140.70
Skeleton	7520.95	609.50	8590.26	723.85	12223.93	1011.74
Internal	1864.94	21.95	1908.91	23.37	3534.27	43.37
Total Phosphorus		716.30		841.49		1210.41

Table VI

PERCENTAGE DISTRIBUTION OF PHOSPHORUS REFERRED TO TOTAL PHOSPHORUS

Composite Samples	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Skeleton	85.09	86.04	83.58
Lean and Fat	10.62	9.94	11.62
Internal	3.06	2.78	3.59
Hair and Hide	0.91	0.88	0.83
Horn and Hoof	0.18	0.19	0.18
Blood	0.14	0.17	0.20
	100.00	100.00	100.00

In Table VI and Curve 4 the percentage distribution of phosphorus referred to total phosphorus shows that most of the phosphorus is found in the bone and that the Group I animal, the fattest animal, has the smallest percentage of its phosphorus in the bone. The lean and fat composites show the Group I animal to have relatively the greatest percentage of phosphorus referred to total phosphorus. The Group II animal has a smaller percentage of its phosphorus in the internal organs than do the other groups. The per cent of phosphorus referred to total phosphorus in the hair and hide sample is less in the Group I than in the Group III animal. Since the growth of the skeleton of Group I is relatively less than Group III the per cent


Curve 4
SHOWING THE PERCENTAGE DISTRIBUTION OF PHOSPHORUS REFERRED TO TOTAL PHOSPHORUS
PLATE B — FOR SALE BY THE UNIVERSITY CO-OPERATIVE STORE, COLUMBIA, MO.
DEPARTMENT OF ENGINEERING, UNIVERSITY OF MISSOURI
0
88%
548 III
552 II
557 I
Blood
Horn and Hoof
Hair and Hide
Internal
Lean and Fat
Skeleton

of phosphorus might be equal to or perhaps even greater than Group I and yet the per cent of the phosphorus as compared with the total phosphorus content of the animal might be smaller in Group I.

Discussion of Sulfur Data

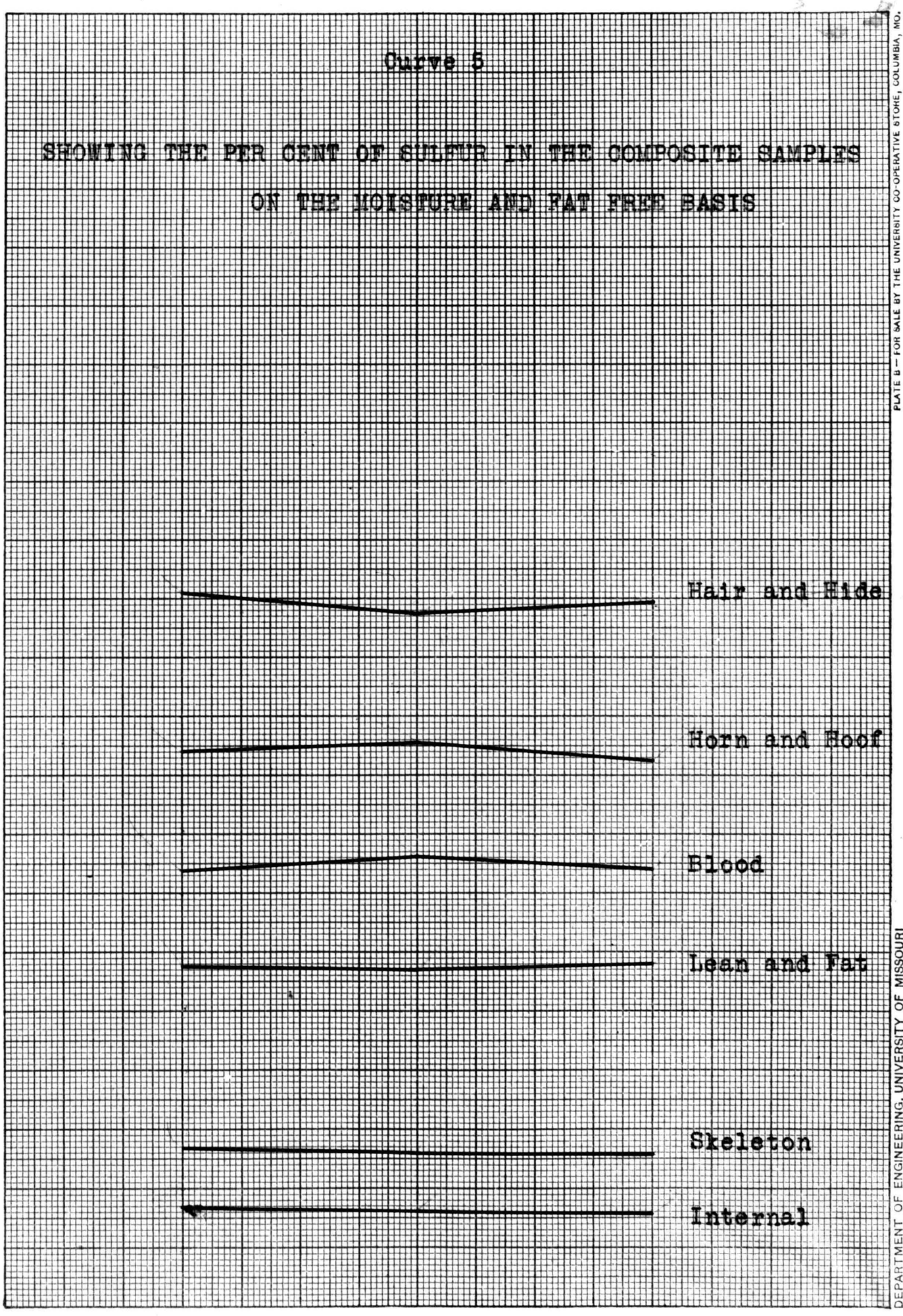
The per cent of sulfur is shown in Table VII and Curve 5--the percentages decreasing in the following order: horn and hoof, hair and hide, lean and fat, internal, blood, and skeleton. The hair and hide shows the lowest sulfur content for the Group II animal, Group I and Group III each containing a higher percentage. The higher per cent of sulfur in the Group III animal would be explained by the fact that Group II and Group I were better nourished animals than Group III. It is a recognized fact that a well nourished animal has a lighter coat of hair /than a poorly nourished animal. The horn and hoof offers only slight variations, Group II being a little higher than Groups III or I. The blood composites of Groups III and I vary only one one-thousandth of one per cent. The composite blood of 552 had undergone slight putrefaction before being dried and can not be considered a normal figure. It shows an increase of 0.18 per cent over Groups I and III in spite of its decomposition changes. With a possible loss of ammonia or carbon dioxide the rise in sulfur content would be easily accounted for. The indications are that the sulfur is not lost from the blood by decomposition. The sulfur

content of the lean and fat samples is very uniform, Groups III and I being slightly higher than Group II. The skeleton and internal composites show Group III to have the highest per cent of sulfur. The composite of the total animal shows Group III to contain a higher per cent of sulfur than either Group II or Group I, Group II being the lowest. The increased sulfur content of the hair and hide of Group III would practically account for the increase of the composite of the entire animal. The percentage sulfur content of all the Group III composites, composite samples from a slow growth animal, indicates a rather general increase of sulfur when compared with a Group II and Group I animal. The sulfur found in each of the entire animals as shown in Table VIII is 155.32 grams for Group III, 167.42 grams for Group II, and 266.04 grams for Group I.

Table VII

SULFUR IN COMPOSITE SAMPLES ON MOISTURE AND FAT FREE BASIS

Composite Sample	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Hair and Hide	1.006	0.854	0.978
Horn and Hoof	1.707	1.769	1.638
Blood	0.680	0.823	0.679
Lean and Fat	0.893	0.877	0.909
Skeleton	0.341	0.286	0.287
Internal	0.837	0.792	0.792
Composite of Total Animal	0.712	0.671	0.702



+0-1%+0-1%+0-1%+0-1%+1-2%+0-1%+

Table VIII

WEIGHT OF COMPOSITE SAMPLES ON THE MOISTURE AND FAT FREE BASIS AND THE WEIGHT OF SULFUR IN THESE COMPOSITE SAMPLES

	Steer 548, Group III		Steer 552, Group II		Steer 557, Group I	
Composite Samples	Weight of Composite Grams	Weight of Sulfur Grams	Weight of Composite Grams	Weight of Sulfur Grams	Weight of Composite Grams	Weight of Sulfur Grams
Hair and Hide	2813.22	28.33	3332.64	28.46	4492.83	43.94
Horn and Hoof	213.28	3.64	254.86	4.51	349.46	5.72
Blood	865.46	5.89	1055.59	8.69	1795.50	12.18
Lean and Fat	8532.50	76.20	9813.73	86.07	15525.43	141.13
Skeleton	7520.95	25.65	8590.26	24.57	12223.93	35.08
Internal	1864.94	15.61	1908.91	15.12	3534.27	27.99
Total Sulfur		155.32		167.42		266.04

The writer has been unable to find any experimental work bearing on the sulfur liberated thru the hide of animals, but finds some very interesting work by Taylor[28] on the cutaneous elimination of sulfur, phosphorus, and nitrogen in the case of two healthy men.

	Grams Eliminated by J in 28 days	Grams Eliminated by D in 45 days	Average Daily Elimination by J Grams	Average Daily Elimination by D Grams
Sulfur	0.798	0.675	0.028	0.015
Phosphorus	0.076	0.096	0.003	0.002
Nitrogen	5.300	7.200	0.190	0.160

Taylor[28] grants that these results are too low in that the hair was not washed and suggests a factor of ten per cent for the sulfur loss. He states in regard to the phosphorus, and the loss of sulfur: ''When one recalls the richness of the secretions of the scalp, and contrasts the area of the scalp with that of the rest of the body, a certain approximation of this loss is permitted. It is quite certain to be larger for the sulfur than for the nitrogen. Probably ten per cent would represent this loss for the nitrogen and twenty per cent for the sulfur. The washings obviously include both elimination and desquamation. When one notes the minimal figures for the phosphorus, one is led to infer that in all likelihood this represents desquamation solely, being derived from the nu-

clei of the desquamated epithelial cells, and that therefore there is no real cutaneous elimination of phosphorus in any form''. From these figures one sees that the elimination of sulfur by a human amounts to about 0.02 grams per day. In an animal where the "secretions of the hair are so rich in sulfur" it is probable that the sulfur elimination many times exceeds this figure.

Table IX and Curve 6 show the relative distribution of sulfur in the composite samples. The lean and fat contains about fifty per cent of the sulfur found in the entire animal, the other composites ranging in descending order with hair and hide, skeleton, internal, blood, and horn and hoof, from 18 per cent to 3 per cent. There is 49.06 per cent, 51.42 per cent, and 53.05 per cent of the sulfur in the lean and fat of Groups III, II, and I respectively. The relative increase in this per cent of sulfur referred to total sulfur is plainly noted. The Group III animal has 3.79 per cent of its sulfur in the blood, while the Group I animal has 4.58 per cent of its sulfur in the blood. The internal and horn and hoof composites change but slightly. The skeleton and hair and hide composites show a decrease in the per cent of sulfur as referred to total sulfur in Group I as compared with the Group III animal. The decrease is 1.74 per cent sulfur for the hair and hide composite and 3.32 per cent of sulfur in the skeleton composite.

There are several possibilities by which we may account

Curve 6

showing SHOWING THE PERCENTAGE DISTRIBUTION OF SULFUR REFERRED TO TOTAL SULFUR

548 III
552 II
557 I

0 — 54%

Horn and Hoof | Blood | Internal | Skeleton | Hair and Hide | Lean and Fat

for the rise and fall in the distribution of sulfur. It is very possible either that the hair of the hair and hide sample of Group III actually contains a higher per cent of sulfur than that of the other groups or that there is more hair, and the relative distribution of this element is raised in this manner, or there may be less low- sulfur-containing connective tissue. Turning to Table VII we see that in Group III the per cent of sulfur in the hair and hide is higher than in Group II or Group I. The very marked rise in the per cent of sulfur in lean and fat of Group I is probably due to the increased mineral matter which we have suggested in the nitrogen discussion and will point out more clearly when we discuss the ash content. There are doubtless several reasons for such an increase. It might be that the hair and hide is not growing rapidly in Groups II and I, but is being filled out and stretched to accommodate the larger body. This is clearly seen to be the case when we look at the weight of moisture and fat free carcass of Groups III, II, and I which are respectively 2813.22 grams, 3332.64 grams, and 4492.83 grams. The respective weights of the carcasses of Groups III, II, and I, on the fresh basis, are 8358 grams, 10532 grams, and 14100 grams.

In Table IX the per cent of sulfur in Group III of the lean and fat sample is 49.06, in Group I the per cent is 53.05 per cent sulfur. This increase in the per cent of the relative distribution of sulfur in the lean and fat is

compensated by the decrease in the per cent of the total sulfur in the hair and hide composite.

Table IX

PERCENTAGE DISTRIBUTION OF SULFUR REFERRED TO TOTAL SULFUR

Composite Sample	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Lean and Fat	49.06	51.42	53.05
Hair and Hide	18.25	17.00	16.51
Skeleton	16.51	14.67	13.19
Internal	10.05	9.03	10.52
Blood	3.79	5.19	4.58
Horn and Hoof	2.34	2.69	2.15
	100.00	100.00	100.00

Discussion of Ash Data

Table X and Curve 7 show the per cent of ash found in the composite samples calculated to a moisture and fat free basis. The skeleton has by far the highest per cent of ash, with over 48 per cent in the Group I composite. All the other composites range between three and six per cent. In the lean and fat composite there is 4.898 per cent ash in Group III, 4.821 per cent of ash in Group II, and 5.014 per cent of ash in Group I. The ash in the blood is less in Group III than in Group I. The presence of adventitious

Table X

ASH IN COMPOSITE SAMPLES ON MOISTURE AND FAT FREE BASIS

Composite Samples	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Hair and Hide	4.007	4.440	3.675
Horn and Hoof	6.016	7.566	5.720
Blood	3.803	4.222	4.198
Lean and Fat	4.898	4.821	5.014
Skeleton	44.294	46.313	48.151
Internal	6.188	6.586	6.559
Composite of Total Animal	18.446	19.190	17.905

Curve 7

SHOWING THE PER CENT OF ASH IN THE COMPOSITE SAMPLES ON THE MOISTURE AND FAT FREE BASIS

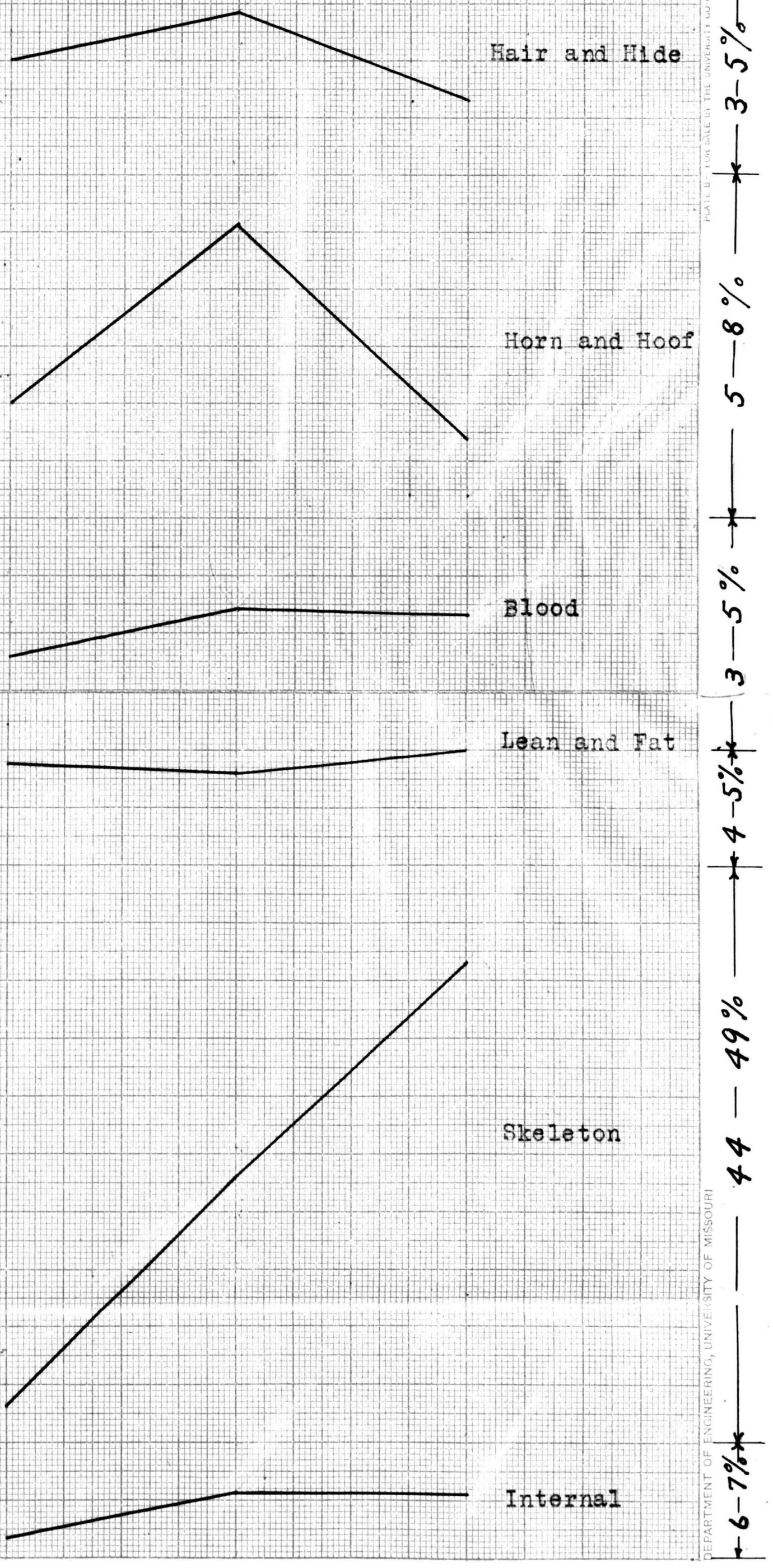


Table XI

WEIGHT OF COMPOSITE SAMPLES ON THE MOISTURE AND FAT FREE BASIS AND THE WEIGHT OF ASH IN THESE COMPOSITE SAMPLES

	Steer 548, Group III		Steer 552, Group II		Steer 557, Group I	
Composite Samples	Weight of Composite Grams	Weight of Ash Grams	Weight of Composite Grams	Weight of Ash Grams	Weight of Composite Grams	Weight of Ash Grams
Hair and Hide	2813.22	112.75	3332.64	147.97	4492.83	165.11
Horn and Hoof	213.28	12.83	254.86	19.27	349.46	19.99
Blood	865.46	32.91	1055.59	44.57	1795.50	75.38
Lean and Fat	8532.50	417.94	9813.73	473.13	15525.43	778.44
Skeleton	7520.95	3331.33	8590.26	3978.44	12223.93	5519.15
Internal	1864.94	115.41	1908.91	125.72	3534.37	231.79
Total Ash		4023.17		4789.10		6789.86

Table XII

PERCENTAGE DISTRIBUTION OF ASH REFERRED TO TOTAL ASH

Composite Samples	Steer 548 Group III o/o	Steer 552 Group II o/o	Steer 557 Group I o/o
Skeleton	82.80	83.07	81.29
Lean and Fat	10.39	9.88	11.47
Internal	2.87	2.63	3.41
Hair and Hide	2.82	3.09	2.43
Blood	0.81	0.93	1.11
Horn and Hoof	0.31	0.40	0.29
	100.00	100.00	100.00

Curve 8

SHOWING PERCENTAGE DISTRIBUTION OF ASH IN THE COMPOSITE SAMPLES REFERRED TO TOTAL ASH

matter in the hair and hide, horn and hoof, and internal composites would easily account for the differences found in those composites. This foreign matter is more pronounced in the hair and hide samples, but it seems reasonable to suppose that it would be found also in the horn and hoof, and internal composites. See Table XI.

Table XII and Curve 8 clearly show the distribution of the ash in the various composites. It is interesting to note that over 30 per cent of the ash of the carcass of an animal is found in the skeleton; about 10 per cent is found in the lean and fat of the carcass, and the remaining 8 to 10 per cent is found in the composite of internal organs, hair and hide, blood, and in the horn and hoof composites. It is interesting to examine Table VI along with Table XII and note how closely the percentage distribution of ash in the carcass represents the distribution of phosphorus.

Discussion of the Ratio of Phosphorus to Ash, Sulfur to Nitrogen, and Sulfur to Phosphorus

Let us now examine Table XIII and see the ratios existing between phosphorus and ash, sulfur and nitrogen, and sulfur and phosphorus. The question of the ratio of phosphorus to ash has often been discussed. Early in the discussion of phosphorus we quoted from the work of Francis and Trowbridge[7] stating that there is no relation between the phosphorus and the ash content of the animal tissue.

Table XIII

THE RATIO OF PHOSPHORUS TO ASH; SULFUR TO NITROGEN; AND SULFUR TO PHOSPHORUS IN THE COMPOSITE SAMPLES

	Steer 548 Group III	Steer 552 Group II	Steer 557 Group I
Ratio of Phosphorus to Ash			
Hair and Hide	1 : 17.292	1 : 20.080	1 : 16.496
Horn and Hoof	1 : 9.869	1 : 11.750	1 : 9.212
Blood	1 : 33.930	1 : 30.528	1 : 31.153
Lean and Fat	1 : 5.492	1 : 5.652	1 : 5.532
Skeleton	1 : 5.466	1 : 5.496	1 : 5.456
Internal	1 : 5.259	1 : 5.381	1 : 5.344
Entire Animal	1 : 5.616	1 : 5.690	1 : 5.592
Ratio of Sulfur to Nitrogen			
Hair and Hide	1 : 15.787	1 : 17.867	1 : 16.006
Horn and Hoof	1 : 9.368	1 : 8.478	1 : 9.458
Blood	1 : 24.246	1 : 18.083	1 : 23.050
Lean and Fat	1 : 16.635	1 : 16.597	1 : 16.597
Skeleton	1 : 24.126	1 : 27.371	1 : 26.695
Internal	1 : 16.582	1 : 17.612	1 : 16.831
Entire Animal	1 : 17.827	1 : 18.344	1 : 17.695
Ratio of Sulfur to Phosphorus			
Hair and Hide	1 : 0.231	1 : 0.259	1 : 0.227
Horn and Hoof	1 : 0.357	1 : 0.363	1 : 0.379
Blood	1 : 0.165	1 : 0.168	1 : 0.199
Lean and Fat	1 : 0.998	1 : 0.973	1 : 0.997
Skeleton	1 : 23.770	1 : 29.480	1 : 28.840
Internal	1 : 1.406	1 : 1.545	1 : 1.550
Entire Animal	1 : 4.613	1 : 5.026	1 : 4.550

This ratio for Group III is seen to be, with the lean and fat sample, 1 to 5.49; with the skeleton composite, 1 to 5.47; and in the case of the internal composite, 1 to 5.26. The composite of the whole animal is 1 to 5.62. The ratio of phosphorus to ash in the case of the blood, hair and hide, and horn and hoof, comes within rather wide limits, their ratios being 1 to 33.93, 1 to 17.29, and 1 to 9.87, respectively. In the Group II and Group I animals the ratio of phosphorus to ash is in rather close agreement with the corresponding composites of Group III. The composite for the entire animal is 1 to 5.69 with the Group II animal, and 1 to 5.59 in the composite for the entire animal in the case of the Group I animal. The average of the ratio of phosphorus to ash in the composites of the entire animal with three different animals is 1 to 5.63.

The ratios of sulfur to nitrogen and sulfur to phosphorus were included to see if there might exist any general ratios of their content in the several composites. The ratio of sulfur to nitrogen ranges between rather wide limits. The closest agreement in these ratios is found in the case of the hair and hide, lean and fat, and the composite of the internal organs. Here we find a variation from 1 : 15.787 in the case of the hair and hide of Group III to 1 : 17.867 in the case of the hair and hide of Group II. The horn and hoof ratio drops to 1 : 8.478 in the horn and hoof of Group II. The blood and skeleton ratios are highest,

and range between 1 : 23.05 and 1 : 27.371.

The ratio of sulfur to phosphorus in all of the samples ranges between 1 : 0.165 to 1 : 1.550, with the exception of the skeleton ratio. The skeleton ratio varies between 1 : 23.77 and 1 : 29.48.

It is interesting to note that there is a fairly constant ratio existing among the same class of tissues. The widest variation in the same class of tissues is found in the skeleton samples. Here we find a variation which ranges between 1 : 23.7 and 1 : 29.5. Many of the samples have a very close agreement in ratios for the same class of tissue. The ratio is little affected by the condition of the animal.

The original data not included in the body of this paper may be found in the appendix.

Part II

WORK ON CYSTINE

Part II

Historical

On account of the important part cystine plays in animal organisms it has been thought fitting to trace rather carefully its historical development as found in the literature. Taurine has been shown to bear a relation to cystine and for this reason some of its history has been given wherever it has a direct relation.

The first indications of sulfur groupings in proteins appeared just prior to 1800, when Crawford[29], in 1797, made an observation that H_2S was evolved during the putrefaction of flesh.

The discovery of cystine was made in 1810 by Wallaston[30] in a urinary calculus. He called this compound cystic oxide.

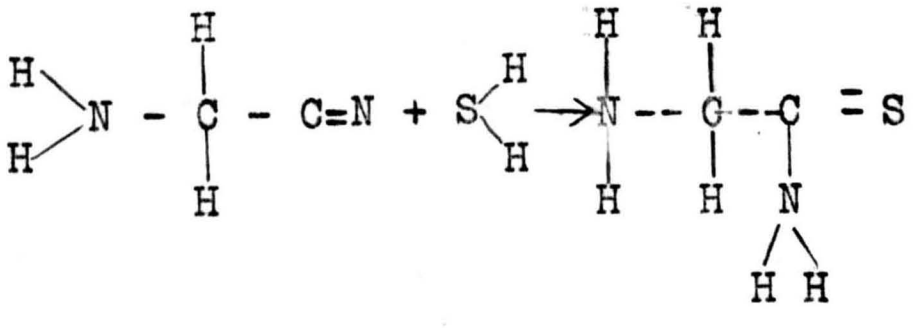
Lassaigne[31] found it under the same conditions in a dog in 1823. Just one year later, in 1824, Tieman and Gmelin[32] made an important discovery of taurine in ox gall. This was given its first elementary analysis in 1833 by Clemarcay[33], whose work was corroborated by Pilonze and Dumas[34], who assigned to taurine the formula $C_2H_7O_5N$. It was left for Redtenbacher[35] in 1846 to prove that taurine contained sulfur and to establish its correct formula--$C_2H_7O_3SN$. In that same year in Liebig's[36] laboratory Fleitmann brought out the important fact that sulfur is

linked in proteins in more than one way. He designated the sulfur as oxidized and unoxidized, according to its reaction on heating with an alkali. His work was confirmed in 1869 by Danielewski[37]. In 1856 Cloetta[38] found cystine in ox kidney, and about this time Berzelius[39] gave it its name. In 1888 Goldmann and Baumann[40] showed that on heating with 10 per cent NaOH 68 per cent of the sulfur was obtained as lead sulfide. Later studies by Suter[41] showed a similar action on the part of proteins. In 1890 Kolz[42] isolated cystine from pancreatic decomposition products of fibrin. This was followed by Emmerling's[43] discovery in 1894 of cystine mixed with tyrosine. On acid hydrolysis of horny substances Drexel[44] who had in 1891 isolated cystine from the liver of a horse, found it in porpoise in 1896. In 1899 Mörner and Embden[45] showed it to be a constant product from the hydrolysis of albumins, and, in 1901, of other proteins.

The first investigations on the constitution of cystine were made in 1871 by Dewar and Gamgee[46], and were followed by numerous others, including Baumann[47], Neuberg[48], Friedman[49], Leowy[50], Erlenmeyer Jr.[51], Suter[52], Fischer and Suzuki[53], Mayer[54], Gabriel[55], Rothera[56], Abderhalden[57], and others, until finally the work of Friedman[58], Emmerling, Jr.[59], and Fisher[60] settled definitely that cystine was di-alpha amino di-beta thio propionic acid.

In 1911 Johnson[61] made a study of cystine and states that " It therefore appears very probable from a consideration of the above evidence that there are other sulfur combinations in proteins besides the cystine group, which can break down, on hydrolysis, with the formation of hydrogen sulfide" . This assumption is based, first, on the fact that the sulfur split off on alkaline hydrolysis as shown by Osborne[24] and others[62] varies greatly with the proteins under consideration. As shown by Buchtala[63] sulfur separates out during hydrolysis with concentrated HCl from some materials and fails to appear on the same treatment of others. Johnson notes the fact that Cohn[64] observed the escape of hydrogen sulfide during the hydrochloric acid hydrolysis of horn shavings, and that Raikow[65] on allowing phosphoric acid to act on unbleached wool observed that sulfur dioxide was evolved. From these and from the observation of other workers[66] Johnson concluded that there were probably other combinations of sulfur besides that found in the cystine group.

Sulfur linkages. Johnson and Burnham[67] in a further study on the linkages of sulfur, studied thioamides and succeeded in preparing thiopolypeptide derivatives by the action of hydrogen sulfide on amino-acetonitrile as illustrated by the following reaction:



This is an example of the formation of a hitherto unknown thioamide of glycocoll prepared by these workers. They found this compound very unstable and going by spontaneous decomposition to the following thioamide:

$$NH_2CH_2CSNH_2 + NH_2CH_2CSNH_2 \text{ -- } NH_2CH_2CS\cdot NHCH_2CSNH_2$$

with the loss of a molecule of ammonia. They "proved" this glycylglycinamide to be an analogue of the carbonyl carbethoxy derivatives described by Fisher[68].

To look at the proteins as these writers have done, as if the sulfur were to substitute an oxygen of an OH grouping, and thereby to get a thio grouping is considering the possible groupings of sulfur in a very practical light. The fact that cystein is really a thio serine in which such a substitution has taken place logically leads one to believe that there should be thiotyrosines and thioprolines.

The writer has not been able to find any other published work relative to possible sulfur compounds other than cystine in the protein molecule.

Experimental

About thirty grams of cystine were prepared from human hair by Folin's[69] method. A good yield was obtained without much difficulty. The only difficulty experienced was in getting the hydrolyzed solution "as clear as water". After recrystallyzing the cystine

three times, perfectly white crystals were obtained. The crystals of cystine were examined carefully after each crystallization to see if there were any tyrosine crystals present, but the writer was unable to find any tyrosine crystals with the cystine. This cystine is the cystine which was used in the course of this experimental work. Ten samples were used in each case.

The composite samples used in Part I were further investigated in Part II to ascertain their cystine content. Six grams of each of the composite samples were hydrolyzed with 20 per cent hydrochloric acid according to the method of Van Slyke[70]. Hydrolysis was continued until the amino nitrogen determined by the Van Slyke method proved to be constant. The period of hydrolysis was 32 hours for all the composite samples excepting the horn and hoof which required 40 hours.

Cystine Content of Samples

The hydrolyzed composites were transferred to 300 c.c. graduated flasks and made up to volume. One hundred c.c. aliquots were pipetted into 400 c.c. beakers and precipitated directly with 7.5 grams phosphotungstic acid. The general procedure was, with such modifications as will be stated later, according to Van Slyke.[71] A sufficiently large aliquot was taken to avoid any further addition of hydrochloric acid. The precipitates were allowed to

stand 48 hours and were filtered thru a Buchner funnel and washed several times with 2.5 per cent phosphotungstic acid which had been acidified with 3.5 per cent HCl. After the precipitate had dried it was transferred to a 500 c.c. separatory funnel and freed from phosphotungstic acid by adding 10 c.c. HCl and a mixture of equal parts of amyl alcohol and ether according to the procedure of Jacobs[72]. The samples all contained much humin and it was necessary to pass the amyl alcohol-ether-phosphotungstic solution thru a Buchner funnel to remove most of the humin in order to get a complete removal of the phosphotungstic acid. The phosphotungstic acid removed, ten c.c.'s of Denis[73] solution were added directly and the solution containing the cystine was concentrated to a few cubic centimeters and transferred to a porcelain evaporating dish and further evaporated to dryness, heated in the muffle at dull redness for ten minutes, taken up with a few cubic centimeters of dilute HCl and brought into solution by heating on the steam bath. This solution containing the oxidized sulfur was filtered and precipitated in the usual way as barium sulfate. The method is Denis'[73] modification of the Benedict[74] method slightly varied as to technique.

Discussion of Cystine Data

Table XIV shows the per cent of cystine precipitated by phosphotungstic acid and the cystine equivalent to the sulfur in the filtrate from the phosphotungstic acid precipitate. The per cent of cystine in the phosphotungstic acid

Table XIV

LOSS OF CYSTINE DURING HYDROLYSIS

Composite	No. of Animal	Group No.	Per Cent Cystine from Phosphotungstic Acid without Corrections for Solubilities 1	Cystine Equivalent of the Sulfur of Filtrate from Phosphotungstic Acid Precipitate 2	Cystine Equivalent of the Sulfur Remaining after Hydrolysis Sum of 1 + 2	Cystine Equivalent of the Total Sulfur before Hydrolysis	Per Cent of Cystine Lost during Hydrolysis
Hair and Hide	548	III	0.5257	0.6817	1.2074	3.7695	67.965
Hair and Hide	552	II	0.5742	0.6796	1.2538	3.1985	60.80
Hair and Hide	557	I	0.6167	0.8935	1.5102	3.6642	58.79
Blood	548	III	0.4331	1.2442	1.6773	2.5477	34.16
Blood	552	II	0.3187	0.9748	1.2935	3.0824	58.03
Blood	557	I	0.4368	1.1730	1.6098	2.5440	36.72
Lean and Fat	548	III	0.4289	2.3578	2.7867	3.3991	18.01
Lean and Fat	552	II	0.4304	1.6455	2.0759	3.2730	36.57
Lean and Fat	557	I	0.4054	1.6314	2.0368	3.4038	40.16
Internal Organs and Offal Fat	548	III	0.4642	1.4376	1.9018	3.1345	39.32
Internal Organs and Offal Fat	552	II	0.4443	1.4151	1.8594	3.4657	46.35
Skeleton	548	III	0.3990	0.8943	1.2933	1.2780	Gain 1.2 o/o
Skeleton	552	II	0.2420	1.4023	1.6443	1.0742	Gain 53.5 o/o
Skeleton	557	I	0.3045	0.9224	1.2269	1.0753	Gain 14.1 o/o
Horn and Hoof	548	III	1.1652	2.5661	3.7313	6.3974	46.17
Horn and Hoof	552	II	1.4964	2.6830	4.1794	6.6241	36.91
Horn and Hoof	557	I	1.7013	3.0689	4.7702	6.1371	22.27

precipitate decreases in the following order: horn and hoof, hair and hide, internal composite, lean and fat, blood, and skeleton composites, ranging from 1.7013 to 0.2420 per cent cystine, taking the same general order as found in the per cent of total sulfur before hydrolysis. The cystine equivalent to the total sulfur of the filtrate varies within wide limits, being in every case larger than the cystine of the precipitate. The sum of the cystine from the phosphotungstic acid precipitate and filtrate is given in the third column. These figures should represent all the sulfur in the sample after hydrolysis, both cystine and non-cystine sulfur. After deducting the solubility correction for cystine, is there any sulfur remaining? If so, what does it represent? Since cystine is, according to Plimmer[75], the only sulfur compound known in the protein molecule and since cystine according to Winterstein[76] is readily precipitated by phosphotungstic acid, it follows that the filtrate from the phosphotungstic acid precipitate (corrected) represents one of three possibilities: (1) inorganic sulfur, (2) transformed cystine sulfur, (3) some unknown compounds of sulfur. We would expect to find transformed cystine since according to Mörner[77] when cystine is treated with 10 per cent hydrochloric acid its specific rotation is changed from -223° to -134° and part of it changed into a "more soluble form". Van Slyke[78] finds that cystine boiled with HCl is destroyed

as shown below:

Hours Boiled with HCl	Per Cent Cystine not precipitated by Phosphotungstic Acid
0	0
8	39
16	41
24	50

The cystine used in this experiment was obtained from a "bladder stone" and was carefully purified. The cystine nitrogen in the filtrate was determined by three methods before hydrolysis and after hydrolyzing 24 hours. Concerning these results, Van Slyke[78] says: "Altho cystine is precipitated as completely as either arginine or histidine the amounts obtained from hydrolyzed proteins fell far short of what would be expected from the sulfur contents. It appeared possible that the cystine is partially destroyed during the hydrolysis. The results in the following table show that this is the case--the cystine is gradually altered during acid hydrolysis into a substance or substances which are not precipitated by phosphotungstic acid." In another paragraph he states in regard to the determination of cystine: "As about half the cystine originally present is altered during the hydrolysis with acids, however, and an amount containing 0.5 per cent of the nitrogen of the protein remains in solution when the bases are precipitated, the amount of cystine obtained by the above method repre-

sents less than half that actually present in the protein."

The sulfur obtained from the phosphotungstic acid precipitate and from the filtrate of the phosphotungstic acid precipitate is given as cystine and compared with the cystine equivalent to the total sulfur. In the total sulfur we would expect to find some inorganic sulfur, but if no sulfur was lost we should find this sulfur in the filtrate from the phosphotungstic acid. We see at once that much of the sulfur is lost during acid hydrolysis and that much of the sulfur is not accounted for in the filtrate from the phosphotungstic acid. Van Slyke[78] suggests that the sulfur is changed to some form not precipitated by phosphotungstic acid and we would expect that if that were the case this sulfur would surely all be accounted for in the filtrate when this filtrate was subjected to strong oxidation. The sulfur is lost in some way and we are led to believe that it is by volatilization during hydrolysis. Later we will take up the possible ways of losing sulfur, but for the present will confine our attention to the data at hand.

As far as the writer has been able to find out the greatest proportion of sulfur found in the animal body is cystine sulfur or cystein sulfur and these two forms are precipitated by phosphotungstic acid[76]. While cystine is the only sulfur found in the protein molecule, cystein, a product of cystine reduction, is also found. The sulfur after hydrolysis should be included in some of the follow-

ing compounds or their derivatives: sulphatides, sulfuric acid as a product of hydrolysis of mucoids or glycoproteins, taurine, chondroitic acid, sulpholipins, and possible sulphocyanites.

We also find in Table XIV the cystine equivalent to the sulfur in the filtrate and precipitate from phosphotungstic acid; the cystine equivalent of the total sulfur of the sample, and the per cent of cystine lost during hydrolysis.

As we stated above, the total sulfur is probably not a true figure of the cystine sulfur, but this comparison will help us to establish the loss of sulfur during hydrolysis. The average per cent of cystine lost during hydrolysis of fourteen samples was 43.01, excluding the skeleton in which case there was more than 100 per cent recovery. The loss on hydrolysis ranges from 18 to 67 per cent. The hair and hide composite is the only sample averaging over 60 per cent loss during hydrolysis. The writer has not been able to find any references other than those cited bearing on the progressive loss of sulfur on continued hydrolysis. The hair and hide, and horn and hoof samples were hydrolyzed longest and they seem to show the greatest loss. The samples were all boiled as uniformly as possible and hydrolysis was discontinued as soon as the amino nitrogen was constant. The skeleton composites failed to lose sulfur beyond the recovery from either the filtrate or pre-

Table XV

CYSTINE EQUIVALENT OF SULFUR

Composite	No. of Animal	Group No.	Cystine Equivalent to Total Sulfur	Non-Cystine Sulfur	Cystine Equivalent to Total Sulfur less Non-Cystine Sulfur
Hair and Hide	548	III	3.7695	0.0000	3.7695
Hair and Hide	552	II	3.1985	0.0000	3.1985
Hair and Hide	557	I	3.6642	0.0000	3.6642
Blood	548	III	2.5477	0.0000	2.5477
Blood	552	II	3.0824	0.0000	3.0824
Blood	557	I	2.5440	0.0000	2.5440
Lean and Fat	548	III	3.3991	1.4642	1.9351
Lean and Fat	552	II	3.2730	0.5567	2.7163
Lean and Fat	557	I	3.4038	0.2304	3.1734
Internal Organs and Offal Fat	548	III	3.1345	0.0000	3.1345
Internal Organs and Offal Fat	552	II	3.4657	0.5530	2.9127
Skeleton	548	III	1.2780	0.0000	1.2780
Skeleton	552	II	1.0742	0.1517	0.9235
Skeleton	557	I	1.0753	0.0000	1.0753
Horn and Hoof	548	III	6.3974	1.3806	5.0168
Horn and Hoof	552	II	6.6241	1.6860	4.9381
Horn and Hoof	557	I	6.1371	2.3342	3.8029

cipitate from phosphotungstic acid. The figure for the per cent of cystine for Skeleton 552 in Column No. 2 is obviously high and makes the sum of 1 + 2 far too high. It is very interesting to note that there is no indication of sulfur volatilized during the hydrolysis of the skeleton.

If inorganic sulfates were present we would expect them to be in the filtrate from the phosphotungstic acid precipitate. Table XV shows the non-cystine sulfur calculated by deducting the cystine present in the filtrate due to the solubility of the same in phosphotungstic acid from the total which is found in the filtrate from the phosphotungstic acid precipitate. Van Slyke established the solubility factor for cystine, which factor when changed from a nitrogen factor to a sulfur factor is the sulfur equivalent to 0.0436 mg. $BaSO_4$ in 200 c.c. of solution. If the filtrate from phosphotungstic acid did not contain 0.0436 mg. $BaSO_4$ the sulfur was all considered to be cystine sulfur and this was calculated from the total sulfur content. This correction in terms of cystine is 1.3225 per cent. On examining this table we note that there is no more sulfur in the filtrate than the amount required for the correction of the solubility of cystine in the case of the hair and hide and blood composites, in two samples of the skeleton and in one of the composites of the internal organs. It is interesting to note that there is an indication of some form of sulfur in the fat and lean after the cystine precipitated by the phosphotungstic acid has been

accounted for. It seems probable that this sulfur might be derived from conjugated proteins which, according to Matthews[79], yield sulfuric acid on acid hydrolysis. The horn and hoof composites show pronouncedly the same indications. We find but a slight indication of sulfur in the filtrate in the case of the skeleton and the internal composites, altho we would expect to find sulfur there derived from the cartilage of the bone as sulfuric acid from chondroitic sulfuric acid[79]. In the case of the skeleton the sum of the cystine in the phosphotungstic acid and the cystine equivalent of the sulfur in the filtrate shows complete recovery. If we calculate the cystine content of these three animals on an assumption that the sulfur is all present as cystine we find in Group III 581.9 grams, II, 626.3 grams, and I, 996.8 grams of cystine.

At the beginning of this investigation the author hoped to be able to find all of the sulfur accounted for in either the filtrate or the precipitate from phosphotungstic acid and then to make a study of ways to determine the sulfur which is present as inorganic sulfates, but on not finding all of the sulfur in either the filtrate or precipitate from the phosphotungstic acid it seemed best to account first, if possible, for the sulfur as yet unaccounted for. There were two possible ways for the sulfur to escape: (1) with the humin, (2) as a volatile compound during hydrolysis.

The following tables according to Abderhalden[80], show the cystine content of a number of keratin substances, all of which are rich in sulfur. The figures for cow's horn and cow's hoof compare very favorably with the figure for horn and hoof in Table XV.

Table XVI

SULFUR CONTENT OF SOME COMMON KERATIN SUBSTANCES

Sample	Per Cent of Cystine
Human Hair	12.98, 14.03, 14.53
Human Nails	5.15
Horse Hair	7.98
Horse Hoof	3.20
Cow Hair	7.27
Cow Hoof	5.37
Pig's Bristles	7.22
Pig's Hoofs	3.17
Cow Horn	6 - 6.80

Humin

Roxas[81] has reviewed the literature and investigated the effects of carbohydrates on the hydrolysis of amino acid and finds 3.1 per cent of the cystine nitrogen destroyed when boiled with 20 per cent HCl + sugar. If the cystine molecule is all destroyed there would also be 3.1 per cent of the cystine sulfur destroyed. This author[81]

states ''There was found evidence to show that the reaction would be different, at least in the case of cystine and tyrosine, if other amino acids were present in the reaction mixture with sugar. If cystine and proline were boiled together in the presence of glucose and 20 per cent HCl a larger amount of cystine nitrogen disappeared in humin formation than when cystine was boiled alone." We find in the animal body a large number of mucins and mucoid bodies, (conjugated proteins) of which, according to Abderhalden[82], there are at least twenty-six different kinds found in various parts of the animal body. Concerning these glycoproteins when hydrolyzed Matthews[83] states: " On decomposition of tendon mucoid by hydrolysis by acids, Levene found that it split into sulfuric acid, galactose, and galactosamine." From the work of Roxas referred to above we would expect to find much humin formed in samples containing mixtures of proteins and more especially proteins containing proline, and in the hydrolysis of samples containing sugars or sugar-like bodies.

Table XVII shows the sulfur precipitated in the humin during the hydrolysis of the fat and lean and of the hair and hide composites of two of the animals.

Table XVII

SULFUR IN HUMIN

Composite	Per Cent of Sulfur in Sample	Wt. of Sample Hydro-lyzed	Grams $BaSO_4$ found in Humin from 2 gram Aliquot	Per Cent of Sulfur found in Humin
Fat and Lean	0.893	6 g.	0.0035	0.0240
Fat and Lean	0.893	6 g.	0.0032	0.0215
Hair and Hide	1.006	6 g.	0.0084	0.0575
Hair and Hide	1.006	6 g.	0.0085	0.0580

The sulfur in the humin of the fat and lean represents 2.55 per cent of the total sulfur; that found in the humin of the hair and hide represents 5.75 per cent of the total sulfur. These two samples show clearly that there is sulfur lost in the humin, but the amount lost there represents only a small percentage of the total loss, an amount which is practically negligible.

The cystine sulfur and the sulfur in the filtrate from the cystine sulfur was not equal to the total sulfur. Was the percentage of sulfur in the hydrolyzed sample equal to the percentage of sulfur in the filtrate and precipitate from phosphotungstic acid? This was found to be true in the case of blood. The filtrate and precipitate from phosphotungstic acid yielded 0.4278 per cent sulfur as compared with 0.4213 per cent sulfur when the sulfur was determined directly upon the hydrolyzed solution.

Sulfur Volatilized During Hydrolysis

When it was found that the lost sulfur was not accounted for in the humin there remained only one known possibility for a loss and that was during hydrolysis, but in order to be assured that this was the case one of the hair and hide composites was hydrolyzed only eight hours and a determination of sulfur in its humin was also run. The sum of the sulfur in this hydrolyzed sample and the sulfur in its humin amounted to 0.8518 per cent as compared with 1.006 per cent of sulfur in the original sample and the loss 0.1488 per cent of sulfur. This was the shortest period of hydrolysis for a hair and hide sample, even shorter than the time required for constant amino nitrogen content, and the indication was that the sulfur was being given off in some volatile compound during hydrolysis.

Mörner[84] states that " traces of alpho thio lactic acid appear to be formed when the heating is prolonged and when sulfur and hydrogen sulfide appear to be formed." Mörner hydrolyzed his samples in closed flasks. Drechsel[85] claimed some of his hydrolyzed solutions gave off an odor of ethyl sulfide. Patten[86] found cystine had been transformed to cystein in some of his hydrolyzed samples.

The writer has been unable to find any data on the quantitative evolution of volatile compounds with the same conditions under which these composite samples were hydrolyzed.

In order to obtain additional proof concerning the sulfur lost during hydrolysis seven of the composite samples were hydrolyzed under the same conditions as those in the first series, except that they were hydrolyzed for 24 hours instead of 30. The time was reduced to 24 hours because the amino nitrogen determinations for 32 hours agreed with those for 24 hours in the first series of samples hydrolyzed. The condensers from these samples were connected with a glass tube leading thru a series of three small Erlenmeyer flasks containing ammoniacal perhydrol solution. Thru the hydrolyzing mass air was gently bubbled which led all escaping gases thru the series of Erlenmeyers containing the ammoniacal perhydrol. This was continued thruout the 24 hour hydrolyzing period and the sulfate in the collecting flasks was determined with the results shown in Table XVIII.

All of these samples show a loss of sulfur in a volatile form which is oxidized to sulfate, but the amount collected is not commensurate with the amount lost from similar composite samples on previous hydrolysis as indicated by the total sulfur determinations. The volatile matter from hair and hide and fat and lean composites gave the test for hydrogen sulfide when passed thru lead acetate solution. These two samples were given only sufficient aspiration to prevent the inside pressure from forcing out the hydrolyte thru the air inlet. The sample of the internal composite was hydrolyzed further for a total of 92 hours at the end of which time it contained 0.3124 per cent sulfur as compared with 0.836 per cent sulfur in the unhydrolyzed sample. This shows a loss of 62.6 per cent sulfur.

Table XVIII

SULFUR VOLATILIZED DURING HYDROLYSIS

Sample Taken	Grams Hydrolyzed	Grams $BaSO_4$	Grams Sulfur in 0.2 gms.	Sulfur Volatilized Moisture and Fat Free Basis o/o	Sulfur in Sample Before Hydrolysis o/o	Sulfur Lost During Hydrolysis o/o
Cystine	0.2	0.0042	0.000578	0.2890	23.6	1.225
Cystine	0.2	0.0062	0.000851	0.4255	23.6	1.830
			Gms.S.in 6 G.			
Composite Internal Organs	6.0	0.0087	0.001190	0.0287	0.836	0.343
Hair and Hide	6.0	0.0135	0.0018537	0.0321	1.006	0.319
Blood	6.0	0.0095	0.001310	0.0252	0.680	0.371
Fat and Lean	6.0	0.0087	0.001190	0.0243	0.893	0.272
Fat and Lean	6.0	0.0116	0.00159	0.0321	0.893	0.359

How can we account for this small per cent of volatilized sulfur when the indications point so strongly toward a much larger loss during hydrolysis? In order to form hydrogen sulfide the samples must first be reduced to cystein and then to hydrogen sulfide. In order to collect the evolved gas in the determination of the hydrogen sulfide liberated from the samples in Table XVIII a current of air was bubbled thru the hydrolyte. According to Matthews and Walker[87] " Cystein oxidizes itself spontaneously by atmospheric oxygen at 20°-22°C at a rapid rate, passing over into cystine." Plimmer[88] also states concerning some work by Fischer and Raske " by oxidizing the resulting cystein by drawing in a current of air thru the solution they obtained cystine."

Since cystein is readily changed to cystine by bubbling air thru the solution all cystein formed would immediately be changed back to cystine and there would be no volatilization of sulfur. It is quite probable that in the case at hand the air was not aspirated thru the solution fast enough to oxidize all the cystein which was formed and that some of this cystein was further reduced to hydrogen sulfide. Assuming this to be true, the hydrogen sulfide formed would represent only a small per cent of the amount which would be formed were air not aspirated thru the solution. The writer was unable to verify these results by making additional collections of the evolved hydrogen sulfide in

perhydrol on account of his inability to obtain additional perhydrol solution; however, another series of determinations was made in which aliquot parts of the hydrolyte were taken at intervals with the plan to make total sulfur determinations upon these aliquot parts and find out if the sulfur content decreases during the period of hydrolysis.

Table XIX

RESULTS SHOWING LOSS OF SULFUR DURING HYDROLYSIS

Sample Taken	Per Cent Sulfur in Sample	Hydrolized 12 hrs. Per Cent Sulfur	Hydrolized 28 hrs. Per Cent Sulfur	Hydrolized 48 hrs. Per Cent Sulfur
Cystine	23.6000	23.6200	23.6100	23.4600
Hair and Hide Composite	1.0060	0.6978	0.5641	0.5254
Lean and Fat Composite	0.8930	0.3135	0.3110	0.3079
Lean and Fat Composite	0.7590	0.3213	0.3002	0.2142
Blood Composite	0.6800	0.3473	0.3122	0.3068

Table XIX shows for all composites a decrease in the sulfur content of the hydrolyte which shows without doubt that sulfur is being lost during hydrolysis. The hair and hide, lean and fat, and blood composites show a definite loss of sulfur during hydrolysis. The loss is greatest

during the first twelve hours of hydrolysis. In the case of the cystine sample we find practically no loss of sulfur from the solution during the 28 hours of hydrolysis.

Table XX

RESULTS FROM ASPIRATION OF FOUR SAMPLES

Sample Taken	Per Cent Total Sulfur	A Air Aspirated thru During Hydrolysis o/o Sulfur	B Air Not Aspirated thru During Hydrolysis o/o Sulfur
Composite of Internal Organs		0.5436	0.4790
Composite of Internal Organs		0.5238	0.4476
Composite of Internal Organs		0.6246	0.3446
Average	0.8366	0.5635	0.4237
Cystine + Protein		0.1090	0.1111

In Table XX are given two samples: (1) a composite of internal organs, (2) a sample of pure coagulated protein to which 0.1 gram of cystine had been added before hydrolyzing. The samples were hydrolyzed 24 hours and their sulfur content was determined. The procedure for both samples was exactly the same except that air was bubbled thru the solutions of Column A while no air was bubbled thru the solutions of Column B. The average of the figures for per

cent sulfur in the internal composite samples of Column A is 0.1398 per cent higher than the average of the figures for Column B. The pure protein with cystine added to it seems to remain practically the same in both Column A and Column B.

In the above table there was a loss of sulfur in both the aspirated and unaspirated samples. The losses are, however, greater where the samples were not aspirated with air. The per cent of sulfur after hydrolyzing 28 hours is slightly less, but is entirely within the limits of analytical error. It must be borne in mind that the loss of cystine reported by Van Slyke[77] (50 per cent on 24 hours hydrolysis) is not an actual loss as would be experienced if the sulfur were liberated as some volatile compound. The cystine was changed to some form not precipitable by phosphotungstic acid. The sum of the sulfur in the phosphotungstic acid precipitate and the sulfur in the filtrate from the phosphotungstic acid precipitate showed a complete recovery of the sulfur in the original sample of cystine*. The writer would state that the fact that the cystine is changed to a form not precipitated by phosphotungstic acid would not preclude the possibility that a pure protein would be changed in the same way. On the contrary, the writer found that in every case excepting the skeleton samples the sulfur was actually lost from the system and not "transformed to some more soluble form". In the case of the

*This is obvious when we inspect Van Slyke's[77] data.

skeleton sample this seems to be the case, in as much as all of the sulfur was found in the precipitate or in the filtrate from phosphotungstic acid.

From the foregoing work on the loss of sulfur by volatilization it seems that a suggestion regarding the hydrolysis of samples for cystine determinations would not be out of place. The writer finds a decided loss of sulfur by volatilization before the hydrolysis is complete. Aspiration of air thru the sample could be regulated to lessen this loss of sulfur and possibly to prevent it entirely. If the oxidizing action of the air which is aspirated thru the sample is not sufficient to prevent reduction of the cystine to a volatile compound it is possible that a small amount of oxygen drawn thru the solution with this air would accomplish this desired result. If it is found impossible to bring about the hydrolysis of a protein without losing forty, fifty, or sixty per cent of the cystine sulfur it would seem there is little value in the cystine determination.

Summary

Part I

I. The poorest fed animal has the highest per cent of nitrogen of the three animals.

II. The medium fed animal in most cases has the lowest per cent of nitrogen.

III. The proportions of total nitrogen found in the various samples are roughly the same for all three animals.

IV. In general, the better the condition of the animal the greater is the relative amount of nitrogen found in the lean and fat, internal organs, and blood, and the smaller the amount found in the skeleton and hair and hide.

V. The condition of the animal has, with one exception, no effect upon the per cent of phosphorus in the sample. In the case of the skeleton the heavier and better conditioned animals have a higher per cent of phosphorus than does the slow growth animal.

VI. The proportion of total phosphorus found in the various samples is but slightly affected by condition.

VII. The condition of the animal has but slight effect upon the per cent of sulfur in the various samples. The better conditioned animal has on the average a slightly lower per cent of sulfur than does the poorer conditioned animal.

VIII. In general, the condition of the animal seems to have no effect upon the proportion of total sulfur found in the various samples. Variations in this proportion are much greater for sulfur than for nitrogen and phosphorus. In the skeleton the better the condition of the animal the smaller the proportion of total sulfur found. With the lean and fat sample it is just the reverse.

IX. With most of the samples the better the condition of the animal the greater the per cent of ash. This is very strikingly true in the case of the skeleton, maturity being the cause of the increase. Variations in the hair and hide, and horn and hoof samples are probably due to adventitious matter.

X. In general, the condition of the animal seems to have but little effect upon the relative distribution of total ash found in the different samples.

XI. There is a fairly constant ratio of phosphorus to ash, sulfur to nitrogen, and sulfur to phosphorus in the same composites for different animals. The ratio of phosphorus to ash is practically 1 to 5.5 in the case of the lean and fat, skeleton, internal composite, and the entire animal.

Summary

Part II

I. Every sample analyzed contained cystine.

II. After being hydrolyzed the horn and hoof, lean and fat, clearly show non-cystine sulfur. The internal organs and skeleton probably contain non-cystine sulfur after hydrolysis.

III. The total sulfur of horn and hoof samples calculated to cystine give a figure in close agreement to the amount of cystine reported by earlier investigators.

IV. Sulfur is lost during hydrolysis from every sample except the skeleton. The sulfur volatilized ranges from eighteen to sixty-seven per cent, the average loss being 43.01 per cent.

V. The loss of sulfur from the composite samples during hydrolysis is greatest during the first twelve hours.

VI. There was no sulfur volatilized from pure cystine during hydrolysis.

VII. The humin of the fat and lean, and hair and hide composites contained a small amount of sulfur.

VIII. The aspiration of air thru the hydrolyte lessened the loss of sulfur.

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SHOWING WEIGHT OF NITROGEN, PHOSPHORUS, SULFUR, AND ASH; PER CENT NITROGEN, PHOSPHORUS, SULFUR, AND ASH ON THE MOISTURE AND FAT FREE BASIS

	Weight of Fresh Sample Grams	Weight of Moisture and Fat Free Sample Grams	Nitrogen Grams	Phosphorus Grams	Sulfur Grams	Ash Grams	Moisture and Fat Free Basis Nitrogen o/o	Phosphorus o/o	Sulfur o/o	Ash o/o
Steer 548										
Hair and Hide	8,358	2,813.22	446.82	6.52	28.33	112.75	15.883	0.232	1.006	4.007
Horn and Hoof	435	213.28	34.10	1.30	3.64	12.83	15.988	0.610	1.707	6.016
Blood	4,603	865.46	142.69	0.97	5.89	32.91	16.487	0.112	0.680	3.803
Lean and Fat	39,653	8,532.50	1,267.52	76.06	76.20	417.94	14.855	0.891	0.893	4.898
Skeleton	19,753	7,520.95	618.76	609.50	25.65	3,331.33	8.227	8.104	0.341	44.294
Internal	10,490	1,864.94	258.84	21.95	15.61	115.41	13.879	1.177	0.837	6.188
Composite of Entire Animal	83,292	21,810.35	2,768.73	716.30	155.32	4,023.17	12.695	3.284	0.712	18.446
Steer 552										
Hair and Hide	10,532	3,332.64	508.48	7.37	28.46	147.97	15.258	0.221	0.854	4.440
Horn and Hoof	550	254.86	38.24	1.64	4.51	19.27	15.005	0.643	1.769°	7.566
Blood	5,219	1,055.59	157.09	1.46	8.69	44.57	14.882	0.138	0.823	4.222
Lean and Fat	47,817	9,813.73	1,428.48	83.70	86.07	473.13	14.556	0.853	0.877	4.821
Skeleton	21,302	8,590.26	672.42	723.95	24.57	3,978.44	7.828	8.430	0.286	46.313
Internal	12,485	1,908.91	366.29	23.37	15.12	125.72	13.949	1.224	0.792	6.586
Composite of Entire Animal	97,905	24,955.99	3,071.00	841.49	167.42	4,789.10	12.301	3.372	0.671	19.190
Steer 557										
Hair and Hide	14,100	4,492.83	703.31	10.01	43.94	165.11	15.654	0.222	0.978	3.675
Horn and Hoof	695	349.46	54.11	2.17	5.72	19.99	15.485	0.621	1.638	5.720
Blood	8,952	1,795.50	280.73	2.42	12.18	75.38	15.635	0.135	0.679	4.198
Lean and Fat	91,027	15,525.43	2,261.29	140.70	141.13	778.44	14.565	0.906	0.909	5.014
Skeleton	29,408	12,223.93	936.40	1,011.74	35.08	5,519.15	7.664	8.277	0.287*	48.151
Internal	26,588	3,534.27	471.10	43.37	27.99	231.79	13.330	1.228	0.792	6.559
Composite of Entire Animal	170,770	37,921.42	4,706.94	1,210.41	266.04	6,789.86	12.411	3.192	0.702	17.905

°This sample had started to decompose.
*Sample lost; this is a calculated figure.

COMPOSITION OF VARIOUS PARTS AND OF TOTAL INTERNAL SYSTEM--- FRESH BASIS

	Wt. of Sample Grams	o/o H_2O	Wt. of H_2O Grams	o/o Fat	Wt. of Fat Grams	o/o N	Wt. of N Grams	o/o Ash	Wt. of Ash Grams	o/o P	Wt. of P Grams
Steer 548											
Offal Fat	845		444.24		310.77		12.35		6.08		1.03
Circulatory System	753		528.46		94.73		19.62		6.35		1.18
Respiratory System	1,084		833.41		33.94		31.52		11.99		2.19
Nervous System	526		392.57		58.19		8.61		7.54		1.84
Digestive and Excretory System	7,282		5,649.40		279.35		186.74		83.45		15.71
Internal Organs and Offal Fat	10,490	74.843	7,848.08	7.407	776.98	3.47	258.84	1.10	115.41	0.21	21.95
Steer 552											
Offal Fat	1,784		516.00		1,208.77		16.48		6.78		1.23
Circulatory System	1,056		676.55		219.48		22.88		8.32		1.37
Respiratory System	1,096		853.78		28.65		29.86		13.22		2.25
Nervous System	466		345.18		53.49		7.29		6.38		1.56
Digestive and Excretory System	8,083		5,970.00		704.19		189.78		91.02		16.96
Internal Organs and Offal Fat	12,485	66.970	8,361.51	17.738	2,214.58	2.13	266.29	1.01	125.72	0.19	23.37
Steer 557											
Offal Fat	6,757		942.26		5,640.14		29.39		15.47		1.96
Circulatory System	2,342		1,183.08		903.00		38.64		14.40		2.46
Respiratory System	1,972		1,519.49		103.69		51.23		22.07		4.00
Nervous System	551		399.90		68.52		9.86		7.77		1.86
Digestive and Excretory System	14,966		10,896.51		1,397.14		341.98		172.08		33.09
Internal Organs and Offal Fat	26,588	56.195	14,941.24	30.512	8,112.49	1.77	471.10	0.88	231.79	0.16	43.37

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COMPOSITION OF SAMPLES ANALYZED--Fresh Basis

	Wt. of Sample Grams	0/0 H_2O	Wt. of H_2O Grams	0/0 Fat	Wt. of Fat Grams	0/0 N	Wt. of N Grams	0/0 Ash	Wt. of Ash Grams	0/0 P	Wt. of P Grams	0/0 S	Wt. of S Grams
Steer 548													
Hair and Hide	8,358	65.408	5,466.80	0.933	77.98	5.346	446.82	1.349	112.75	0.078	6.52	0.339	28.33
Horn and Hoof	435	49.982	217.42	0.988	4.30	7.839	34.10	2.949	12.83	0.299	1.30	0.837	3.64
Blood	4,603	81.198	3,737.54	0.000	0.00	3.100	142.69	0.715	32.91	0.021	0.97	0.128	5.89
Lean and Fat	39,653	73.749	29,243.82	4.733	1,876.68	3.197	1,267.52	1.054	417.94	0.192	76.06	0.192	76.20
Skeleton Composite	19,753	46.671	9,218.87	15.254	3,013.18	3.132	618.76	16.865	3,331.33	3.086	609.50	0.130	25.65
Internal Composite	10,490	74.843	7,848.08	7.407	776.98	2.468	258.84	1.100	115.41	0.209	21.95	0.149	15.61
Composite of Entire Animal	83,292		55,732.53		5,749.12		2,768.73		4,023.17		716.30		155.32
Steer 552													
Hair and Hide	10,532	66.823	7,037.80	1.534	161.56	4.828	508.48	1.405	147.97	0.070	7.37	0.270	28.46
Horn and Hoof	550	52.348	287.91	1.314	7.23	7.449	38.24	3.520	19.27	0.304	1.64	0.811	4.51
Blood	5,219	79.774	4,163.41	0.000	0.00	3.009	157.09	0.854	44.57	0.028	1.46	0.166	8.69
Lean and Fat	47,817	70.026	33,484.32	9.451	4,518.95	2.988	1,428.48	0.989	473.13	0.175	83.70	0.180	86.07
Skeleton Composite	21,302	43.802	9,330.71	15.872	3,381.03	3.156	672.42	18.676	3,978.44	3.398	723.95	0.115	24.57
Internal Composite	12,485	66.972	8,361.51	17.738	2,214.58	2.133	266.29	1.007	125.72	0.187	23.37	0.121	15.12
Composite of Entire Animal	97,905		62,665.66		10,283.35		3,071.00		4,789.10		841.49		167.42
Steer 557													
Hair and Hide	14,100	63.123	8,900.48	5.012	706.69	4.988	703.31	1.171	165.11	0.071	10.01	0.312	43.94
Horn and Hoof	695	48.759	338.87	0.960	6.67	7.705	54.11	2.869	19.99	0.312	2.17	0.823	5.72
Blood	8,952	79.943	7,156.50	0.000	0.00	3.136	280.73	0.842	75.38	0.027	2.42	0.136	12.18
Lean and Fat	91,027	58.120	52,905.05	24.824	22,596.52	3.484	2,261.29	0.855	778.44	0.154	140.70	0.155	141.13
Skeleton Composite	29,408	43.235	12,714.65	15.198	4,469.42	3.184	936.40	18.768	5,519.15	3.440	1,011.74	0.119	35.08
Internal Composite	26,588	56.195	14,941.24	30.512	8,112.49	1.772	471.10	0.871	231.79	0.163	43.37	0.105	27.99
Composite of Entire Animal	170,770		96,956.79		35,891.79		4,706.94		6,789.86		1,210.41		366.04

COMPOSITION OF SAMPLES ANALYZED--MOISTURE AND FAT FREE BASIS

	Wt. of Fresh Sample Grams	Moisture and Fat Free Wt. of Sample Grams	% H_2O	Wt. of H_2O Grams	% Fat	Wt. of Fat Grams	% N	Wt. of N Grams	% Ash	Wt. of Ash Grams	% P	Wt. of P Grams	% S	Wt. of S Grams
Steer 548														
Hair and Hide	8,358	2,813.22	65.408	5,466.80	0.933	77.98	15.883	446,82	4.007	112.75	0.332	6.52	1.006	28.33
Horn and Hoof	435	213.28	49.982	217.42	0.988	4.30	15.988	34.10	6.016	12.83	0.610	1.30	1.707	3.64
Blood	4,603	865.46	81.198	3,737.54	0.000	0.00	16.487	142.69	3.803	32.91	0.112	0.97	0.680	5.89
Lean and Fat	39,653	8,532.50	73.749	29,243.82	4.733	1,876.68	14.855	1,267.52	4.898	417.94	0.891	76.06	0.893	76.20
Skeleton Composite	19,753	7,520.95	46.671	9,218.87	15.254	3,013.18	8.227	618.76	44.294	3,331.33	8.104	609.50	0.341	25.65
Internal Composite	10,490	1,864.94	74.843	7,848.08	7.407	776.98	13.879	258.84	6.188	115.41	1.177	21.95	0.837	15.61
Composite of Entire Animal	83,292	21,810.35		55,732.53		5,749.12	12.695	2,768.73	18.446	4,023.17	3.284	716.30	0.712	155.32
Steer 552														
Hair and Hide	10,532	3,332.64	66.823	7,037.80	1.534	161.56	15.258	508.48	4.440	147.97	0.221	7.37	0.854	28.46
Horn and Hoof	550	254.86	52.348	287.91	1.314	7.23	15.005	38.24	7.566	19.27	0.643	1.64	1.769	4.51
Blood	5,219	1,055.59	79.774	4,163.41	0.000	0.00	14.882	157.09	4.322	44.57	0.138	1.46	0.823	8.69
Lean and Fat	47,817	9,813.73	70.026	33,484.32	9.451	4,518.95	14.556	1,428.48	4.821	473.13	0.853	83.70	0.877	86.07
Skeleton Composite	21,302	8,590.26	43.802	9,330.71	15.872	3,381.03	7.828	672.42	46.313	3,978.44	8.430	723.95	0.286	24.57
Internal Composite	12,485	1,908.91	66.972	8,361.51	17.738	2,214.58	13.949	266.29	6.586	125.72	1.224	23.37	0.792	15.12
Composite of Entire Animal	97,905	24,955.99		62,665.66		10,283.35	12.301	3,071.00	19.190	4,789.10	3.372	841.49	0.671	167.42
Steer 557														
Hair and Hide	14,100	4,492.83	63.123	8,900.48	5.012	706.69	15.654	703.31	3.675	165.11	0.222	10.01	0.978	43.94
Horn and Hoof	695	349.46	48.759	338.87	0.960	6.67	15.485	54.11	5.720	19.99	0.621	2.17	1.638	5.72
Blood	8,952	1,795.50	79.943	7,156.50	0.000	0.00	15.635	280.73	4.198	75.38	0.135	2.42	0.679	12.18
Lean and Fat	91,027	15,525.43	58.120	52,905.05	24.824	22,596.52	14.565	2,261.29	5.014	778.44	0.906	140.70	0.909	141.13
Skeleton Composite	39,408	12,223.93	43.235	12,714.65	15.198	4,469.42	7.664	936.40	45.151	5,519.15	8.277	1011.74	0.287	35.08
Internal Composite	26,588	3,534.27	56.195	14,941.24	30.513	8,113.49	13.330	471.10	6.559	231.79	1.228	43.37	0.792	27.99
Composite of Entire Animal	170,770	37,921.42		96,956.79		35,891.79	12.411	4,706.94	17.905	6,789.86	3.192	1210.41	0.702	266.04

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