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Chemical and Physical Changes in the Bones of Fasting Animals

Chemical Engineering

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CHEMICAL AND PHYSICAL CHANGES IN THE BONES OF FASTING ANIMALS

BY

PAUL EVANGEL JOHNSTON

THESIS

FOR THE

DEGREE OF BACHELOR OF SCIENCE

IN

CHEMICAL ENGINEERING

COLLEGE OF SCIENCE

UNIVERSITY OF ILLINOIS

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June 1, 1912

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Paul Evangel Johnston

ENTITLED Chemical and Physical Changes in the

Bones of Fasting Animals.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in

Chemical Engineering

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CHEMICAL AND PHYSICAL CHANGES IN THE BONES OF FASTING ANIMALS.

INTRODUCTION.

There has been considerable experimental work done on bones and a great many articles have been written on the subject in the last fifty years. The bones of the various parts of the organism and of many different animals have ben analyzed. The bones of the elephant, horse, dog, cat, rabbit, dove and man are the most notable. A great deal of analytical work, however, was performed some time ago, when chemistry was less exact than now and quantitative methods less accurate than at present.

Carnot[1] performed the first quantitative determination of fresh bones. It is as follows:

	Human Femur		Ox
	Body of Femur	Head	
$CaPO_4$	87.45	87.87	85.72
MgPO4	1.57	1.75	1.53
CaF_2	.35	.37	.45
$CaCl_2$	.23	.30	.30
$CaCO_3$	10.18	9.23	11.96
Iron Oxide	.10	.13	.13
	99.88	99.65	100.09

Another early experimenter was Milne-Edwards, who analyzed normal bones of the dog and cat. Another early work was that of Von Bibra in 1844. Several parts of the skeletons of dogs of different ages were analyzed. The analyses of the femur of a sixteen year old deer hound and of a three to four year old English dog are given as follows:[2]

	Deer Hound	English Dog
Calcium Phosphate	59.24	51.63
Calcium Carbonate	9.63	12.63
Magnesium Phosphate	1.11	1.74
Salts	.62	.52
Fat	.91	1.14
Ossein	28.49	32.34
Ash	70.60	66.52
Organic Substance	29.40	33.48

Voit[3] experimented on a dove. With a low calcium ingestion the bones lost very little weight. From this experiment he concludes that there is very little change in the bone during starvation. Aeby[4] did some interesting work on fossil elephant bones. Volkman[5] analyzed fresh and dry human bones. Later. Weiske and Weldt[6] analyzed the bones of sheep which had been fed varying amounts of calcium and phosphorus. Their analysis is as follows:

	Sheep I	Sheep II	Sheep III
	Low Phosphorus Per cent	Low Calcium Per cent	Normal Per cent
Organic Substance	32.86	35.68	33.18
Inorganic Substance	67.14	64.32	66.82
Calcium	34.76	34.00	66.82
Magnesium	.62	.63	.70
Phosphoric Acid	26.45	26.18	26.79

Weiske[7] carried on some interesting experiments on rabbits. The blood, flesh, bones, and other parts of the organism were analyzed. In this experiment was studied the effect of an addition of calcium, strontium, and magnesium to a low calcium but high phosphoric acid food, upon the skeleton.

Calcium was often reported as $3CaO.PO_4$, $CaO.CO_3$, $CaCl$ and CaF while magnesium was reported as $3MgO.P_2O_5$. The bone was considered to be a mechanical mixture and not a definite chemical compound. Its composition was early recognized to be dependent to some extent on the nature of the diet.[8] Graffenberger[9] gives the following analyses:

	Per Cent Ash
Rabbit Bone	71 - 72
Horse "	63.8
Dog "	66.01
Cow "	66.35

Osteomalaceous and normal bones of man and the horse have been analyzed by McCrudden[10]. As they may be of interest, they are given here as follows:

	Man		Horse	
	Normal	Osteomalacia	Osteomalacia	Normal
CaO	15.44	28.85	20.09	33.48
MgO	.57	.14	.50	.11
P_2O_5	12.01	19.55	16.35	23.66
S	.55	.14	.55	.11

Other analyses have also been made, but very little really has been done to determine the changes which take place due to fasting. Chossat [3] found that the bones of a starving dove lost seventeen per cent of their original weight. He also found that the skeleton of a cat lost fourteen per cent of its original weight. In 1907 Wellman[11] analyzed the skeleton of a normal and starved rabbit and found that the starving bone was rich in water and poor in fat but that the composition of the bone did not change to any noticeable extent. In 1893 Gussmitta[12] reported a very importand experiment to determine the changes in the bone of a fasting dog. In

TABLE I.

Total Wt. of Dry Bones	Organic Substance		Inorganic Matter Total	Fe Phosphate	$CaCO_3$	$Mg_3(PO_4)_2$	$Ca_3(PO_4)_2$	Sol. Salts & Bases
	Ossein	Fat						
Grams	o/o	o/o	o/o	o/o	o/o	o/o	o/o	o/o
Right								
11.91	32.95	6.12	60.93	.16	5.78	.94	53.88	.17
Left								
10.38	34.81	2.02	63.16	.13	5.20	.78	56.84	.21

this experiment, the lower portion of the normal animal's left hind leg was amputated and the bones examined histologically and chemically. The individual was then subjected to a fatal fast and the corresponding bones of the other side were analyzed as before. On the following page are tabulated the results of his experiment.

It will be noticed upon examination of the data that there was a greater decrease of organic substance than inorganic substance, due chiefly to a loss of fat - since the ossein tends to maintain a constant relation to the inorganic matter. The greatest loss was in $CaCO_3$, due to the solubility of $Ca(HCO_3)_2$ - the more soluble substances being removed first. There was an increase in the water content and a decrease of all the organic and inorganic constituents. The experiment of Gussmitta is of particular value because it is upon the bone of a single animal under normal and fasting conditions.

EXPERIMENTAL.

It was the object of this investigation to determine the alterations, if any, which took place in the bones of animals when such animals were subjected to a fasting regime. It was proposed to study the physical as well as the chemical characteristics. The bones of seven animals were examined, four of them dogs of different ages which had fasted for varying intervals, one a fasting fox, and the remaining animals being normal dogs for comparison. Two of the fasting dogs were "repeated fasters". For example Dog No.1, a female about one to two years old, had been the subject of two fasts - the first being fifteen days in length whereas the second extended thirty days.[13] Each fast was begun with the animal at nitrogen equilibrium. The other "repeated faster" was Dog No.3. This was the fasting dog Oscar, an adult Scotch collie, holding the distinction of fasting over one hundred days

on two separate occasions. The first fast was one of one hundred seventeen days after which the beast was fed carefully and after regaining his normal weight was fasted again for an interval of one hundred four days.[15] Dog No.2 was an adult dog which fasted forty eight days,[16] whereas Dog No.4 was an adult fox terrier, which fasted fourteen days. The weights of these dogs at the beginning and end of the fast and the percentage lost in body weight are given in the following table:-

Dog No.	Body Weight (Kg.)		Length of Fast	Loss in Body Weight
	Beginning of Fast	End of Fast	Days	Per Cent
1	3.54	1.91	30	46
2	13.62	6.42	48	53
3	26.33	9.76	104	63
4	5.80	3.61	14	38
Fox	5.12	4.42	13	13

The bones subjected to examination were in each instance the left femur. These bones with the exception of Dog No.4 had been kept from sixteen to eighteen months and were thoroughly air dried. Some of the fat had been lost from the normal bones.

Physical Examination.

The bones were thoroughly cleaned and scraped free of the periosteum. They were then put in a small power-driven testing machine and their ultimate tensile strength obtained by loading as a beam in the middle. The formula $\frac{M}{S} = \frac{I}{C}$ was used to compute the tensile strength. The formula is a well known one in mechanics and a sample calculation is given on the next

page.

Normal Dog No. I.

$$I = \frac{1}{64}\pi\left(d^4 - d_1^4\right)$$

W = Load = 233

l = length under strain

$$\frac{M}{S} = \frac{I}{c}$$

$$S = \frac{Mc}{I}$$

$c = \frac{1}{2} d$

d = large diameter

$d = .4$

d_1 = small ..

$$M = \frac{1}{4} Wl$$

$$d^4 - d_1^4 = .0233$$

$d_1 = .22$

$$\frac{\pi}{64} = .0492 \qquad S = \frac{233 \times 3 \times 2}{4 \times .0492 \times .0233} = 30{,}480$$

lb. per sq. in.

A large canvas screen was placed around the machine so that the peices scattered by the breaking of the bone would not be lost. The crookedness of the bones made it difficult to keep them from turning or slipping. Also, because bones crush a little before the maximum load is applied, the neutral axis was changed and there was an unavoidable error in the value of c. Assuming no error to be in c due to the shifting of the neutral axis, the most accurate method of finding the value of $\frac{I}{c}$ is the measurement of the photograph in which the cross section is enlarged a definitely known number of times. The area is irregular and may be found by means of calculus. Possibly a more accurate method of obtaining the maximum tensile strength is by putting the ends of the bone in blocks of plaster paris and breaking them by tension rather than bending. Before putting the bones in the machine, the longest straight (or nearly straight) length on the bone was chosen and lines were drawn around the bone with an indelible pencil at the ends of this length

and one exactly in the middle. The bearings of the maching were at these points and were covered with thin peices of blotting paper to prevent slipping. Care was also taken to have the bone exactly in the center of the machine.

The cross sections were made as nearly as possible to the point of rupture and as often as possible from the piece in which the longitudinal section was made. A smooth neat cross section was very difficult to obtain. It was found that a very fine-toothed bracket or hack saw is the proper instrument for making such cross sections. The saw must be drawn very slowly and carefully across the bone to prevent shattering and ruining the section. The bone was blocked in a vise with small pieces of wood . A piece of paper was placed under the vise to catch most of the dust and flying particles.

Chemical Examination.

After photographing the sections, the bones were broken into a few smaller pieces and dried five hours in a hydrogen oven at 105°, the hydrogen oven being used to eliminate the possibility of oxidation. The hydrogen oven consisted of an ordinary air oven resting on its door which was fastened firmly by wires to a table and which rested on an electric hot plate. Hydrogen was led in from a Kipp generator through a water trap and then through a large $CaCl_2$ U-tube. The inlet was bent upward on the inside of the oven so as to direct the stream of hydrogen upward and not disturb the bone in the open dishes.

After heating for five hours, the dishes were placed in desiccators to cool and then weighed. The bone was placed in a large hardened filter paper, crimped so as to hold its maximum amount of material. The cone was closed with cotton,(previously extractedand free from fats), placed in a large Soxhlet extractor,(in the bottom of which was a layer of fat-free cotton for a safety filter,) and the bone was extracted with absolute or anhydroue ether for five hours. The extract was collected in weighed

flasks. Anhydrous ether was used to insure a moisture free residue.

It was the desire of the experimenter to determine the phosphorus and nitrogen content of the ether extract and from the ratio of the per cent nitrogen and phosphorus (1.78 N to 3.8 P) in lecithin calculate the amount of lecithin and other nitrogenous constituents (if any) in the marrow. In order to do this the extract (which had been weighed after removing the ether by evaporation) was dissolved in ether and made up to 250 c.c. Aliquot parts, (50 c.c.) were placed in a Kjeldahl flask, most of the ether driven off, H_2SO_4 (30 c.c.) and $CuSO_4$ added, the contents digested till colorless and nitrogen determined by the Kjeldahl method. For the phosphorus determination , the rest of the ether- solution was placed in a Kjeldahl flask and the ether driven off on a steam bath. Nitric acid (30 - 50 c.c.) was added, a reflux condenser attached and the flask gently heated. After a moment's heating a violent reduction took place, brown fumes and liquid shooting out of the top of the condenser - thus losing the determination. It was found by experimentation upon sheep bone extract that this violent action will not take place if all the ether is driven off. The oxidation of fats with nitric acid is a long tedious operation. Evaporating once or twice to dryness seems to assist the HNO_3 in decomposing the substance. The organic substance to be disintegrated was usually heated with HNO_3 with a reflux condenser attached to the mouth of the flask for about a half hour. It was then removed to the Kjeldahl digestion hood and the contents of the flask slowly evaporated to dryness. They were then treated with more HNO_3 (30 c.c.) or aqua regia and heated for some time. If the acid did not seem to "take hold" of the organic matter it was again carefully evaporated, to dryness, and treated with more acid. When the solution was no more colored brown from organic matter, it was diluted, cooled, almost neutralized with NaoH solution and made up to 250 c.c.

Aliquot parts were then taken for analysis.

The residue from the ether extraction was ground in an iron mortar, passed through about a fifty-mesh sieve and thoroughly mixed. The grinding took from four to twelve hours for the different bones and the writer would suggest that a power mortar could be used to advantage. That the iron from the blood in the bone and from the mortar does not interfere with the accuracy of the results was determined by dissolving the $Mg_2P_2O_7$ of five crucibles in HCl and determining the iron colorimetrically with KSCN. Iron in bones was investigated in 1871 by Plugge,[17] KSCN being used to detect traces of iron. In the $Mg_2P_2O_7$, the iron expressed as FeO was found to be .0001 g.

Samples of the ether extract residue were treated with HNO_3 in the same way as the extract and one gram samples of bone were ashed, dissolved in hot HNO_3, almost neutralized, cooled and made up to 250 c.c.

Nitrogen was determined in the ether extract residue by digesting .5g. or 1g. of substance in H_2SO_4 and determined in the usual way by the Kjeldahl method.

The ash was determined according to standard methods.[18]

Phosphorus was determined by taking three 40 c.c. portions of the solution prepared for analysis, diluting with about thirty cubic centimeters of water, adding about twenty grams ammonium nitrate. After the ammonium nitrate was dissolved, 50 c.c. ammonium molybdate[19] were added and the beakers placed in a pan of warm water and digested at 65 ° for one hour. The solutions were stirred when the molybdate was added and two or three times during the digestion. After filtering, the precipitates were washed with a dilute solution of ammonium nitrate slightly acidified with HNO_3,[20] until 5 c.c. of filtrate gave no test for chlorides or until no test was obtained for molybdenum with $K_3Fe(CN)_6$. The Filtrate was redigested for one half hour at 65° with the addit-

ion of 5 - 10 c.c. ammonium molybdate. The precipitate was then dissolved with equal parts NH_4OH and hot water, the solution being received in the same beaker in which the precipitation was made. The filter was washed several times with ammonium hydroxide and hot water, the solution nearly neutralized with HCl and allowed to cool. (The ammonium phospho-molybdate was used as an indicator, the appearance of its yellow color indicating an acid reaction.) Ten cubic centimeters of magnesia mixture were added drop by drop from a burette at the rate of one drop per second and the solution stirred during the process. After standing fifteen minutes, one third the volume of strongNH_4OH (.96 sp. gr.) was added, the solution stirred and the beakers covered and allowed to stand over night. The precipitate was filtered into ashless filters, washed until a few c.c. of the wash water gave no test for chlorides and the precipitates allowed to dry. Only 2.5 per cent ammonia was used as wash liquor. The magnesium ammonium phosphate was burned with the filter paper in weighed crucibles, which had been previously heated in a muffle for one hour. The filter paper was first slowly volatilized by heating at a low temperature on an asbestos gauze, care being taken not to allow the gases to catch fire and carry off some of the ash mechanically. The crucibles were then placed in a muffle , heated for at least an hour and cooled in a dessicator. The ash was thoroughly ground with a smooth , rounded, dry glass rod, care being taken to exclude all drafts from the room and to catch all the ash falling outside the crucible on a glazed paper and to remove all the ash from the rod with a small camel's hair brush. The crucibles were returned to the muffle and heated for three to four hours, when the ash was perfectly white. The crucibles were then cooled in a ~~des$icator~~ desiccator containing fresh $CaCl_2$ and having a stop-cock whose inside end was bent upwards so that the inrush of the air would not disturb the magnesium pyrophosphate.

Calcium and magnesium were determined according to McCrudden's method[21] which is as follows:-

"1. The solution is brought to a volume of 75 to 150 c.c. Concentrated ammonia water us added drop by drop till the elution is just alkaline, as shown either by the appearance of a precipitate of calcium and magnesium phosphates or by the use of an indicator(alizarin or litmus paper).

"2. Concentrated hydrochloric acid is added drop by drop until the solution is just acid, as shown either by the disappearance of the precipitate or by an indicator. In the presence of iron an indicator must be used.

"3. Ten drops of concentrated hydrochloric acid (sp.gr. 1.20), approximately equivalent to 10 c.c. of $\frac{N}{2}$ HCl, are added.

"4. Ten cubic centimeters of 2.5 per cent oxalic acid are added.

"5. The solution is boiled until the precipitated calcium oxalate is coarsely crystalline, and then an excess of 3 per cent ammonium oxalate is slowly added to the boiling solution and the boiling continued until the precipitate is coarsely crystalline.

"6. The solution is cooled to room temperature and 8 c.c. of 20 per cent sodium acetate added.

"7. The solution is allowed to stand over night.

"8. The calcium oxalate is filtered off on small ash-free paper and washed free from chlorides with .5 per cent ammonium oxalate solution.

"9. The precipitate is washed three times with cold distilled water, filling the filter about two-thirds full and allowing it to drain completely before adding more. A hole is made in the paper and the calcium oxalate washed into the flask. The volume of the fluid is brought up to 50 c.c. and 10 c.c. of concentrated sulphuric acid are added. The oxalate is titrated immediately with standard potassium permanganate."

"The filtrate containing magnesium is evaporated almost to dryness after addition of 20 c.c. of contentrated nitric acid. When almost dry, and fumes of nitric oxide no longer come off, 10 c.c. of concentrated hydrochloric acid are added and the solution again evaporated almost to dryness. The solution is diluted to about 80 c.c., nearly neutralized with ammonia and then cooled. .... Enough sodium acid phosphate is added to precipitate the magnesium, if enough is not already present, and a slight amount added in excess. Ammonia is then added drop by drop with constant stirring until the solution is alkaline, and then enough more added slowly and with constant stirring to make the solution contan one-fourth its bulk of dilute ammonia (specific gravity 0.96). The solution is allowed to stand over night. Next day it is fltered and washed free from chlorides with alcoholic ammonia solution (1 part alcohol, I part dilute ammonia, 3 parts water). The precipitate with filter paper is incinerated slowly and carefully with good supply of air to prevent reduction in the usual manner. "

The writer of this thesis found a slight precipitate in the solution after evaporating the solution containing magnesium to dryness, so the solution was filtered before precipitating the magnesium. Microcosmic salt was added to the cold slightly acid solution instead of sodium hydrogen phosphate, the solution stirred and allowed to stand for at least a half hour. It was found that the magnesium did not form a precipitate until the solution was strongly alkaline, when McCrudden's method was followed exactly. After standing with occasional stirring the precipitate formed in the neutral or slightly acid solution and the solution was made strongly alkaline as above, with frequent stirring. It was also found that when fairly concentrated (say 50 - 80 c.c.) solutions of the bone were used in the determination of calcium, the values for magnesium were lower than in the more dilute solutions. It is therefore recommended that the solutions be diluted to large

volumes (say 300 c.c.) before precipitating the calcium.

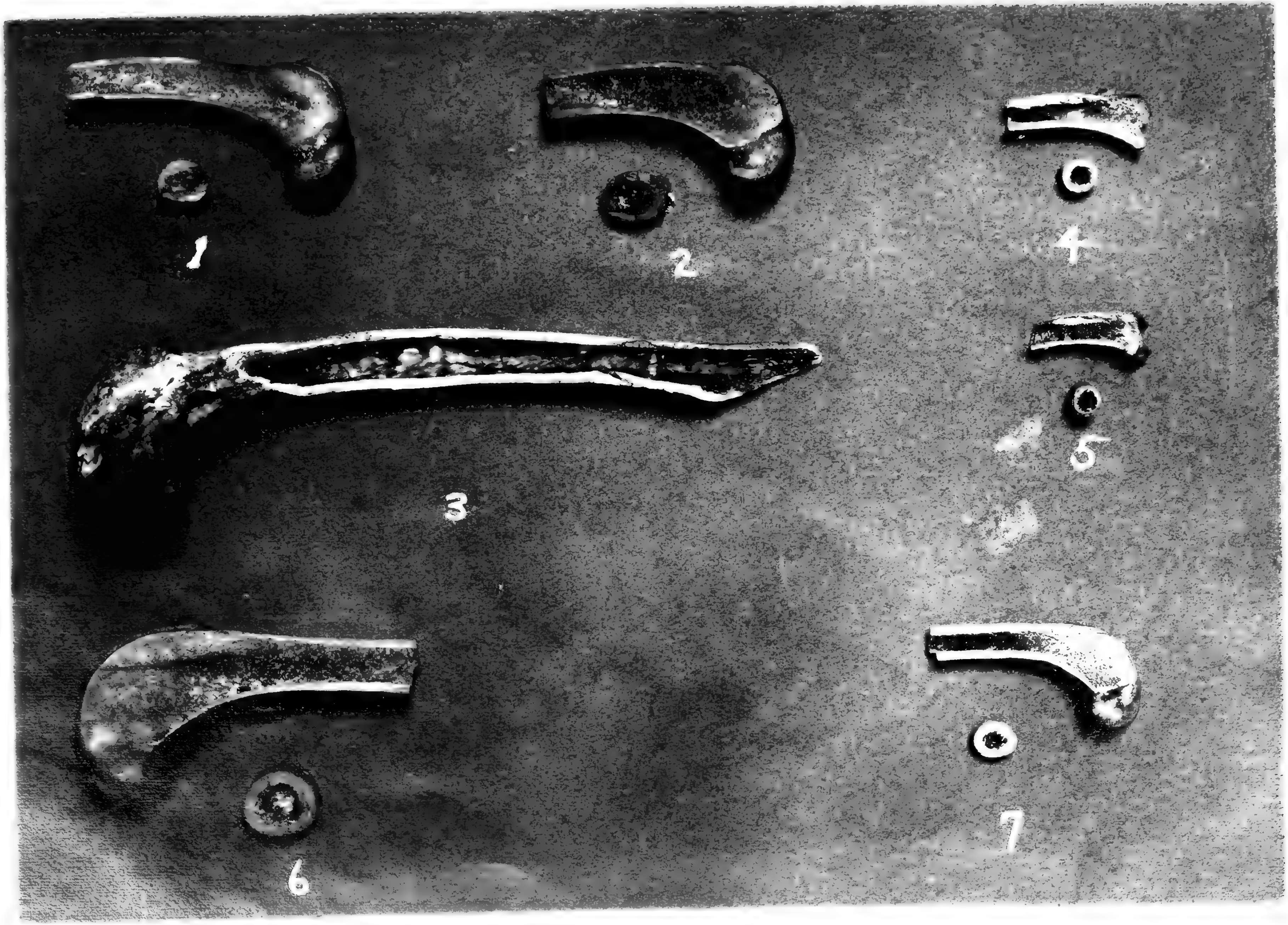
Aron has a very simple method for determining calcium in organic substances.[22] It is especially adaptable when the experimenter does not wish to determine the presence of other elements.

DISCUSSION.

Photographs of sections (both cross and longitudinal) of the bones examined are reproduced in Plate I. The point emphasized upon glancing at this plate is the depletion of the marrow shown in the fasting bones. This point is demonstrated particularly well in the case of No. 3, the femur of a dog which fasted forty eight days. We would call particular attention to sections No. 6. These were made from the femur of "Oscar" after a fast of a hundred four days. If we compare these sections with those of the normal and fasting bones of the other animals we will observe that they more nearly retain their normal appearance than do the other fasting bones. This fact is very significant when it is recalled that these sections(6) were made from the femur of a dog which had been subjected to a hundred four day fast whereas the other sections (3,4,5 and 7) were made after fasting intervals ranging from thirteen to forty eight days. The contrast between sections 6 and 7 is particularly striking representing, as they do, fasting intervals of 104 and 14 days in length respectively.

It will be observed that the walls of the fasting femurs (except 6) are much whiter than those of the normal bones. This is probably due among other things to the lower fat values. Section 6 which is nearer the normal bones in ether extract value is seen to be midway between the fasting and normal bones, in so far as the physical characteristics of its walls is concerned.

Our data from the breaking tests indicate that the ultimate


1
2
4
3
5
6
7

KEY TO PLATE I.

Femurs of Normal and Fasting Animals.

1,2 Normal dogs I and II

3 Fasting dog 2

4 Fasting dog 1

5 Fasting fox

6 Fasting dog 3
"Oscar"

7 Fasting dog 4.

tensile strength of dog bones is 25,000 to 35,000 pounds per square inch. Very few breaking tests have been made on bones and these mostly on bones of the hog. Christopher found the ultimate tensile stress for hog femur[23] to be about 12,000 pounds per square inch. The ultimate tensile strength of wood being 10,000 per square inch and of cast iron 20,000 pounds per square inch, the surprising conclusion must be reached that the dog's femur is probably at least twice as strong as good timber and one and a quarter times as strong as cast iron.

The data from the breaking tests reported by Christopher furnish evidence against a low protein diet. It is there shown that the pigs fed a low protein diet "do not develop as strong or as healthy a bone as pigs on a medium or high protein diet." Henry[24] has made a very comprehensive study of the influence of diet upon the strength of the bones of swine. Among other points he demonstrated that the feeding of bone meal caused an increase of over 50 per cent in the strength of the bones as compared with similar bones from an animal nourished solely on corn. When wood ashes were added to the diet, the strength of the bones was found to be nearly double that of the bones of the corn-fed animals. Shelton and Cottrell[25] have also studied the influence of diet upon the breaking strength of bones. Their data however show no pronounced differences in this respect. Experiments made by Carlyle[26] on growing hogs indicate that the inclusion of beef meal in proper proportion in the ration of such animals produced a marked increase in the strength of the skeleton. In this same connection it was noted that the feeding of a ration rich in corn meal caused the growth of a skeleton which was abnormally weak.

Further experiments upon the influence of diet upon the breaking strength of bones have been made by Burnett.[27] He observed that the bones

were 50 per cent stronger when a ration consisting of corn meal (90o/o) and bone meal (10 o/o) was fed than they were when the dietary consisted solely of corn meal. A series of interesting experiments showing the influence of a diet poor in phosphorus upon skeletal strength have been made by Hart, McCullom and Fuller.[28] Pigs were used as experimental subjects. It was found that the bones of the skeleton of the low-phosphorus pigs had a breaking strength only about one-third as great as was that of the bones from the skeletons of pigs fed a larger phosphorus quota. They conclude that phosphorus was withdrawn from the skeleton to make good the deficiency of this element in the food. When phosphorus was thus withdrawn they found that this removal was accompanied by the withdrawal of calcium. It is of interest that the phosphorus and calcium were removed in the proportions found in calcium phosphate. Aeby had previously observed that the bones of goats were rendered weak when the diet of the animal was low in phosphorus.

In connection with a series of important investigations to determine the effect of diet upon the growth and development of swine Forbes[30] has found interesting data. In two of the final tests attention was accorded the question of skeletal growth and development. It was noted that the swine fed on corn or low phosphorus rations possessed skeletons which were comparatively weak. The actual tensile strength of the bones was greatest for those animals nourished on bone flour and "bran extract". The low values for calcium and protein in the diet are given as the principal reason for the low tensile strength of the corn-fed pigs. It was found in the case of a single animal that the addition of lecithin to the diet aided satisfactory skeletal development.

The bone analyses were on the fat- and water-free bone and are calculated to that basis. The ether extract is based upon the original dry

T A B L E II.

CHEMICAL COMPOSITION OF BONES

Animal	Length of Fast (Days)	Ether Extract	Ash	Nitrogen	CaO	MgO	P O	Tensile Strength Lb. per Sq. In.
		o/o	o/o	o/o	o/o	o/o	o/o	
Normal Dog I		14.7	55.79	4.5	30.72	2.1	11.68	25,000
" " II		20.4	62.56	4.2	35.90	1.6	12.60	-
Fasting Dog 1	30	-	62.31	4.95	32.8	1.5	12.76	35,000
" " 2	48	-	60.18	4.75	34.4	.8	12.56	-
" " 3	104	13.7	61.50	4.6	33.3	.8	12.80	30,000
" " 4	14	0.1	61.56	4.1	33.1	.9	12.90	-
" Fox	13	2.6	58.90	5.45	25.5	.1	10.41	-

bone. By referring to the data tabulated in Table II, it will be seen that there was a pronounced variation in the quantity of ether extract secured from the different fasting bones, as well as from bones of different normal animals. The difference between the ether extract values for the bone of fasting dogs No. 3 and 4 is of particular interest. It will be remembered that No. 3 was the animal which fasted 104 days whereas No.4 was subjected to a fast of only two weeks. After the two weeks fast the bones of Dog No. 4 contained only 0.1 per cent ether extract whereas the bones of Oscar contained 13.7 per cent ether extract at the end of the 104-day fast. The finding of such a high value for ether extract in the bone of this dog is a rather remarkable observation, and serves to further emphasize the wonderful power possessed by him of conserving his resources. At the autopsy upon this animal surprisingly large deposits of subcutaneous fat were in evidence as were also large deposits in the abdominal region, particularly about the kidney. In connection with the high ether extract of the bone of this dog the conclusion of Trowbridge and Woodman[31] in connection with the fat content of the bones of poorly nourished steers is of interest. They say, " In steers which have suffered from insufficient nutrition for a long period, the fat may be nearly all resorbed from the skeleton."

The normal value obtained by Gussmitta was 6.1 per cent, a value only about 1/2 - 1/3 of our normal value whereas his fasting value was 2.0 per cent a figure which corresponds fairly well with our value for the fasting fox. Gussmitta's dog weighed 6 kg. and fasted 28 days. After the experiment here reported was completed the writer determined the ether extract in a fresh normal bone (femur) and found it to be 26 per cent of the original dry bone. It is probable therefore that the two normal bones listed in Table II lost a portion of their fat during the interval preceding their analysis.

The percentage of ash in the normal bones is seen to vary from 55 to 62 per cent the latter value being somewhat similar to that secured by Gussmitta (60.9 per cent). There was apparently no pronounced change in the ash values of the fasting bones which can serve to differentiate them from normal bones. The ash values for the four fasting dogs were very uniform ranging from 60.18 per cent to 62.31 per cent. The fox bones were found to be lower in ash than those of any of the fasting dogs, the value being 58.9 per cent. It is interesting to note that some of these ash values were from the bones of growing dogs. According to Aron[32] the skeleton of a poorly fed growing dog grows and increases both in size and mass, at the expense of the other parts of the body, especially the flesh. The ash of the skeletons of Aron's normal dogs was 3.2 - 5.4 per cent of the body weight, whereas the value for poorly fed dogs was 7.8 per cent. The ash of the bone of the fox was below that of all the fasting bones but above that of one normal bone. Von Bibra[2] found the compact bone of a wolf to contain 60.9 per cent ash and the spongy part to contain 50.3 per cent ash, while Gussmitta gives 60.9 as the per cent ash of his normal dog bone.

The calcium values for the normal bones vary considerably as was the case with ether extract and ash. In Normal Dog I 30.7 per cent CaO was present whereas the value was 35.9 per cent in Normal Dog II. The CaO values for the bones of the four fasting dogs were exceedingly uniform the percentages being 32.8, 33.1, 33.3 and 34.4. This close agreement between the calcium values for the different fasting bones irrespective of the length of the fast is very significant and will be discussed more at length in a later paragraph.

As was the case with the ash the CaO value for the fox bones (25.5 per cent) was much lower than that for the fasting dog bones. One of the normal dog bones was also low in CaO, yielding but 30.7 per cent. It will be observed in this connection that low CaO values are accompanied by low P_2O_5 values.

The fact that the low CaO value in the bone of Normal Dog I is accompanied by high MgO and that the high CaO in the bones of Normal Dog II is associated with low MgO, is an observation not in accord with reported observations of other authors.[12,33] This is in accordance with McCrudden's findings,[10] as shown by the analyses on page 3 of this thesis. In the case of osteomalacia the magnesium has decreased from the normal while the calcium has increased.

The values for MgO in the normal dog bones ranged from 1.6 per cent to 2.1 per cent, whereas the percentages for the fasting bones were all lower than these. The surprisingly close agreement between the MgO values for Dogs No. 2, 3, and 4 is of interest. These values are 0.8, 0.8 and 0.9 respectively. It is significant that these uniform MgO values are associated with CaO values which are also uniform. This relationship between Ca and Mg has been observed in other connections. For example Forbes[30], who has carried on some valuable nutrition experiments on swine, says: "A peculiar relationship between the action of calcium and magnesium in the body requires that a definite proportion between the quantities present be maintained. ..... An excess of magnesium occassions the liberation of calcium in quantity sufficient to counteract the effects of this excess magnesium." The extremely low value (0.1 per cent) for MgO in the bones of the fasting fox is interesting. It may be noted that this low value for MgO is associated by low CaO, low P_2O_5, low ash and high nitrogen.

It will be noted from an examination of the P_2O_5 values that those values for the fasting dogs are very uniform. This uniformity is all the more impressive when the varying length of the fasting periods is taken into consideration. The actual per cents for the four dogs were 12.56, 12.76, 12.80 and 12.90. The possible significance of this pronounced uniformity will be mentioned again in a later paragraph.

The P_2O_5 value for the bones of the fasting fox was very much lower than the similar values for the fasting dogs i.e., 10.41 as against 12.56 to 12.90 per cent.

So far as nitrogen is concerned an examination of the data will indicate that there were no pronounced variations among the bones of fasting dogs or between fasting and normal dogs. The greatest variation was between the bones of Dog No. 1(4.95 per cent) and Dog No.4 (4.1 per cent). The bones of the fasting fox were somewhat richer in nitrogen than were any of the other bones.

When the data from the analyses of the bones are considered as a whole several significant points are apparent. In the first place the wide variation in the percentage composition of the bones of the two normal dogs presents itself. It has been found by experiment that many factors serve to influence the development of bone. For example in connection with our discussion of experiments on the tensile strength of bones many of these points have been emphasized. In addition to these Aron[34] found that a decrease in the sodium ingestion accompanied by an increased potassium intake caused subnormal skeletal development. Desgrez and Zaky[35] found lecithin feeding to have a beneficial influence upon the growth of the skeletons of such animals as dogs, rabbits and guinea pigs. Lawes and Gilbert[36] in their classical investigations demonstrated that the relation between the ash content of the bones of their experimental animals and the ash content of the rest of the body was dependent upon the fatness of the animal. Voit[37] produced bones with a low tensile strength by feeding animals a diet poor in calcium. He furthermore produced a condition simulating rachitis by means of such a diet. Forster[38] also found that bones had a low calcium value when the food did not contain the proper quota of this element. Weiske[7] also studied the

influence of varied dietary conditions upon the bones and in particular their calcium content. Cook[39] fed organic and inorganic phosphorus to rabbits and produced an increase in both these forms of phosphorus in the bones. Aron and Sebauer[40] and Baginsky[41] have both shown a condition simulating rachitis to follow a low calcium ingestion. In certain experiments upon sheep Weiske and Wildt[6] failed to observe any alterations from the normal in the composition of the bones of animals fed diets low in calcium and phosphorus. Zalesky[8] furnishes further evidence that the nature of the bone is altered by recourse to dietary variation. Aron[32] has noted that if a young dog be denied sufficient food to cause normal growth that the skeleton grows at the expense of other parts of the body in particular the musculature. Investigations of the type outlined above serve to indicate the pronounced influence exerted by dietary changes upon the composition of the bones. In the case ~~of the case~~ of the two normal dogs utilized in the present study the animals were fed a similar diet for a short interval preceeding the time their bones were taken for analysis. However this interval was probably not sufficiently long to alter to any appreciable degree the composition of their bones at the time the animals were put upon the experimental diet. The pronounced variations in the analytical data obtained from the bones of these two normal dogs we may therefore infer was due to their previous dietary habits.

The question of age must also be taken into consideration in this connection. The animals under discussion were evidently adult dogs, yet we were unable to determine very exactly what their relative ages were. Experiments by Voit[37] on dogs and by Brubacher[42] on children indicate that increase in age is accompanied by an increased ash and decreased water content of the bones. Graffenberger,[9] observed that the water content of rabbits' bones also decreased with the age of the animal. The influence of age is also

emphasized by Milne-Edwards[43] in the case of the cat, dog and man. For example the femur of a new born kitten contained 4.55 per cent $CaCO_3$ whereas in the bones of a kitten two months old the value was increased to 7 per cent. Similar results were obtained in the case of dog bones and skeletal variation with age was also demonstrated for man.

The question of the influence of species upon the composition of the bones has been but little studied. According to Carnot[1] the relative proportions of CaP04 and $CaCO_3$ are very uniform in the different parts of the same bone and of different bones in the same animal as well as of the bones of animals of different species. According to Peterson and Soxhlet[44] the cartilage of the shark contain over 90 per cent of sodium chloride whereas the CaO value is reduced to 0.4 per cent. Hiller[45] found the inorganic substance of the bones of birds to vary from 32.84 to 52.06 per cent according to the nature of the bone. Düring[46] has also determined the composition of the bones of the bird. He found the humerus to contain the highest per cent of inorganic matter. McCrudden[10] found horse bones to contain about 23.5 per cent of P_2O_5 a value nearly double that found by us in our dog bones. Zalesky[8] analyzed the bones of the hen and cat and studied the influence of diet.

In our own experiment the variation in the composition of bone as influenced by species is quite marked. It will be noted that the fox bones gave an ash value of 58.9 per cent as against 60.18 - 62.31 per cent for the bones of the dog and that furthermore each constituent of the ash so far as determined i.e., CaO, MgO and P_2O_5, were present in smaller percentage than in the case of the dog. The variation in calcium is especially pronounced, being 25.5 for the fox and 32.8 - 34.4 for the dogs.

So far as making a direct comparison between the composition of the normal bones and those of the fasting animals is concerned we can arrive at no definite conclusions by such means. This is due to the fact that the fast-

ing values, are for the most part, midway between the values as determined for the normal bones. In view of this wide variation in the composition of normal bones it is evident there is but one method of experimentation which will yield conclusive results. That method entails the examination of normal and fasting bones <u>from the same animal</u>. Such methods are now being employed in this laboratory for the elucidation of this and other associated problems.

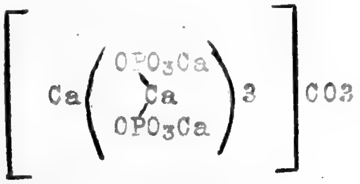
When we examine closely into the comparative composition of the dry, fat-free bones of the fasting dogs we note a striking uniformity. This uniformity is particularly impressive when we compare the data from Dogs 3 and 4. Here we have animals which fasted 104 and 14 days respectively. Notwithstanding the great discrepancy in the period of inanition the analytical data are wonderfully uniform,- in fact excellent duplicates.

~~No~~ How are we to interpret the uniformity of our fasting data in the light of the notable lack of uniformity in our normal data? We have already stated that the variation in the composition of the normal bones may have a dietary basis.

Our data apparently indicate that a fasting interval two weeks in duration is accompanied by some slight readjustment of the relationships of the inorganic constituents of the bone causing the establishment of a definite "fasting mimimum" in the bone structure. This basal structure is not capable of further alteration no matter what the length of the succeeding fasting interval may be. We may consider then that the period of two weeks serves to reduce the irregularities in bone composition, due to the previous dietary habits of the animal, to a common basis. This basal structure of the fasting bone is probably a definite chemical complex. This is indicated by the definite relationship existing between the phosphorus and calcium for example. The ratio of P_2O_5 : CaO is found to be 1 : 2,56 for Dog No.1,

1: 2.60 for Dog No.3 and 1 : 2.56 for Dog No.4.

In this connection the experiments of Gassmann[47] are of interest. He shows that the relation between Ca, PO_4 and CO_3 is the same for normal as for rachitic bones. On the basis of this fact he suggests that the following complex is present in bone;-



$$\left[Ca \left(\begin{matrix} OPO_3Ca \\ \rangle Ca \\ OPO_3Ca \end{matrix} \right)_3 \right] CO_3$$

In any event, however, our data do not indicate that fasting causes any marked changes in the chemical composition of the bones. This finding is in full agreement with the conclusions of Wellman.[11] This investigator found the composition of the fat-free dry substance of rabbits' bone underwent but slight changes during fasting.

CONCLUSIONS.

1. The bones of normal dogs vary considerably in composition, the variations being due principally to age and dietary habits. Because of this fact the only way to satisfactorily investigate the changes due to inanition is to study the normal and fasting bones, from the same animal.

2. The tensile strength of normal and fasting bones is very similar, being about 25,000 pounds per square inch. In either case the tensile strength is more than twice as great as that of good timber and one and a fourth times as great as that of cast iron.

3. The bones of a fasting fox were found to vary decidedly in composition from those of fasting dogs.

4. The length of the fast seemed to have no influence upon the ultimate composition of the inorganic portion of the bone. This was shown by the uniformity in the composition of the bones of dogs fasting 104 and 14 days respectively.

respectively.

5. The nitrogen content of fasting bones (dogs) is not markedly different from that of normal bones. The nitrogen value for fox bones is slightly higher than that for dog bones.

6. The mineral onstituents of bones are present in rather uniform proportions. This is shown by the ratio 1 : 2.56, 1 : 256 and 1 : 2.60. This indicates that the basis of bone is a definite chemical structure and not a mixture.

7. The femur of a dog after a 104-day fast was found to contain 13.7 per cent ether extract whereas the femur of a dog fasting only two weeks contained but 0.1 per cent.

8. The composition of the dry, fat-free bone of fasting dogs apparently undergoes no marked variations from normal values.

9. A fast of two weeks reduces the inorganic substance of a bone to a definite level which is uninfluenced by further fasting.

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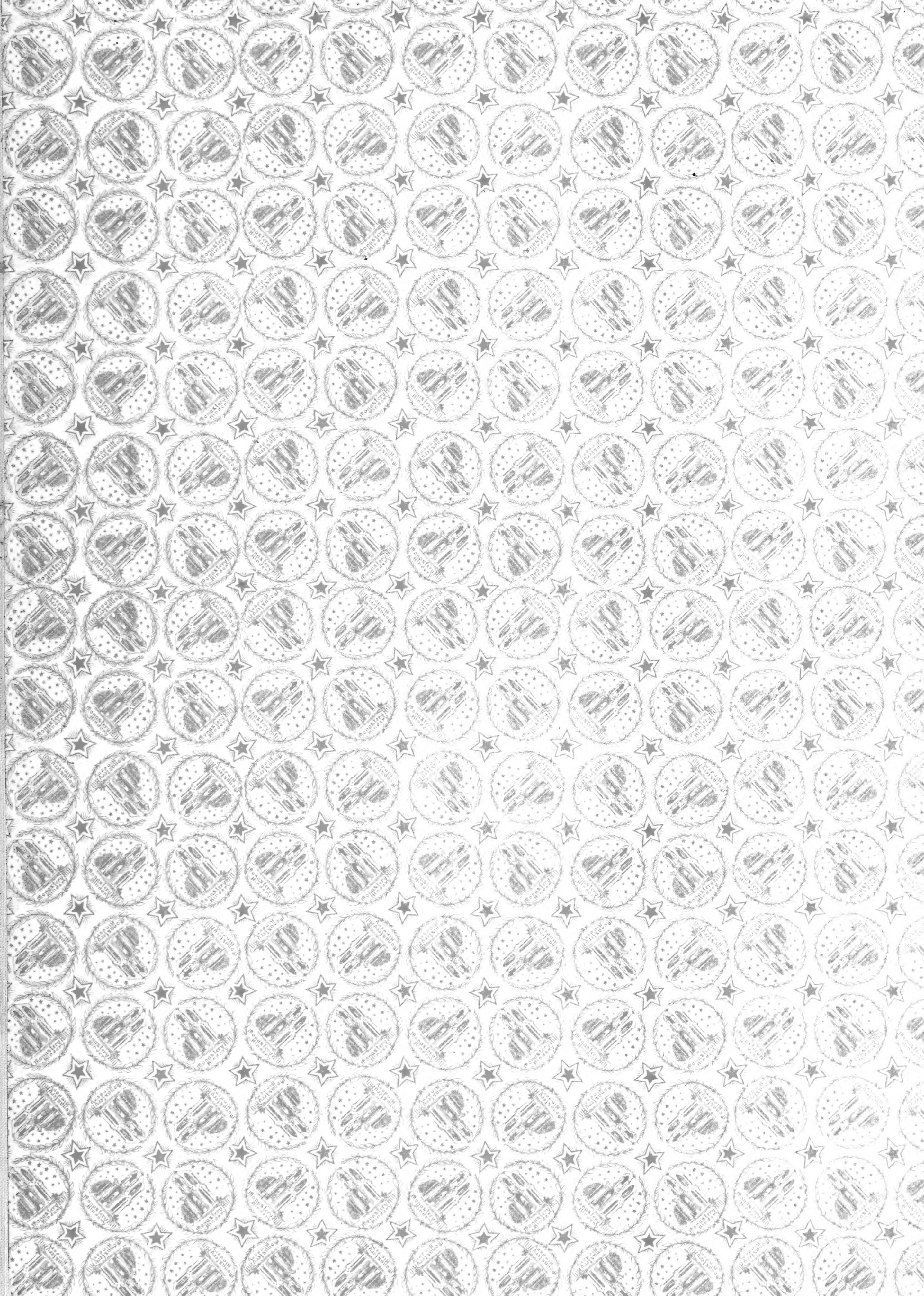
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