

Resumen por el autor, H. E. Radasch.

La determinación del tanto por ciento de substancia orgánica en el hueso.

El tanto por ciento de substancia orgánica en el hueso compacto es 32 a 33 por ciento, según los autores. De la inspección de la literatura general no se desprende el método empleado en la determinación de dicha substancia orgánica. Con el objeto de determinar el tanto por ciento real y hallar, a ser posible, el método empleado por los primeros observadores, el autor ha llevado a cabo varios experimentos de diversa naturaleza. Después de preparar cuidadosamente trozos del fémur, tibia y fíbula, pesó una serie de trozos, que se calcinaron después, pesando el producto de esta operación. La pérdida de peso indica la cantidad de substancia orgánica incinerable que forma parte del hueso compacto.

Entre los veinte a sesenta años de edad, el tanto por ciento medio hallado es 40.75. En el gato adulto el tanto por ciento de peso en del hueso joven es 38.32, mientras que en el conejo (dos tercios del crecimiento total) el tanto por ciento medio es 38.90. Mediante otros métodos, la humedad y las substancias solubles en el alcohol y el éter fueron eliminadas, fijando la cantidad de contenido orgánico fijo. La cantidad media de humedad durante el periodo comprendido entre los veinte y sesenta años es 8.42%, y la relación 7 de la substancia orgánica fija y el hueso seco es solamente 34.92%. La cantidad media de substancia soluble en el éter es 9.27 por ciento; la relación media de la substancia orgánica fija y el hueso extractable es 31.34%. Parece sin embargo que el peso tipo debe ser el del hueso joven, y si se acepta esto, la cantidad media, de substancia orgánica contenida en él es 40.75 por ciento.

THE DETERMINATION OF THE PERCENTAGE OF THE ORGANIC CONTENT OF COMPACT BONE

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In considering the chemical composition of bone we are told that it consists of two main substances intimately commingled, viz., earthy and animal substances. The former comprises the following substances, according to Cunningham's Anatomy ('14):

	<i>per cent</i>
Calcium phosphate.....	53.23
Calcium carbonate.....	7.32
Calcium fluoride.....	1.41
Magnesium phosphate.....	1.32
Sodium chlorid.....	0.69
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	68.97
Organic material.....	31.03
	<hr/>
	100.00

The organic substances comprise fats and ossein.

According to Piersol, who quotes Berzelius, the inorganic material comprises 67.3 per cent, while the organic material consisting of gelatin and blood-vessels constitutes 33.3 per cent. Schaefer, Prudden (Ref. Handbook of Medical Sciences), Sobotta, all give the same as Piersol, apparently having accepted the same source as their guide. Gray's Anatomy ('18) gives the organic content as from 67 to 68 per cent and so the inorganic constitutes 33 to 32 per cent.

We are further told by Schaefer that the animal material, improperly called cartilage of bone, differs from cartilage physically and chemically. It is much more flexible and softer and upon boiling the bone yields mainly gelatin. He concludes that

the animal material closely resembles white fibrous and areolar tissues in that it consists mainly of collagen.

Normal bone is hard, rigid (to a certain extent), tenacious, and also elastic. The earthy materials contribute to its hardness and rigidity, while the organic material gives bone its tenacity and elasticity. With the last characters in mind, we can readily understand some of the results of fractures. Although we are not told so, we naturally conclude that the foregoing percentages and characteristics apply to compact bone and to the adult type. This being agreed, we naturally would consider that the bones of the young and adolescent would contain a greater percentage of organic material and, therefore, a lower percentage of inorganic substance. This chemical difference, therefore, makes a physical difference to the effect that the bones of the young should, theoretically, be more elastic and tend less to fracture under the same proportionate strain than that of the adult; that the writer believes the surgeon will admit. In the case of ultimate fracture, however, we might expect a different result than in the adult, and consequently we find the green-stick fracture pertains to youth entirely and does not occur in the adult. This is due to the higher percentage of organic substance in the bones of the young. This will be shown actually in the succeeding data.

We are told that by subjecting the bone to an open fire (calcining) the organic substance is burned out, leaving a white, brittle, chalk-like substance that preserves its original shape, but with the loss of about one-third of its weight. This porous cast is easily broken, so apparently the substance which gives tenacity has been removed. We might naturally infer from this that in old age there is a reduction of organic substance, for it is known that in old individuals fractures occur more easily than in those in the prime of life; also repair is less rapid and less satisfactory in old age. We would believe, then, in the elderly, that there is a reduction of organic substance that causes the bone to yield more quickly to strains. Yet Rusby (Ref. Handbook of the Medical Sciences) tells us that as age advances there is a diminution in the mineral constituent of bone and the

organic element is slightly increased. This should make the bone somewhat more tenacious and less prone to fracture—just the reverse of actual experience. He states that this increase is due to the replacement of bone by enlarged blood-vessels and marrow, so that there is, in reality, a reduction of fixed organic material. Yet in those bones examined in this work no reduction was noted, but rather an increase, so that theoretically the bone should not be weakened in old age as it naturally is. There is apparently a thinning or diminution of the actual bony layer of the long bone, and this accounts partly for the readiness of the fracture.

Hoppe-Seyler is quoted (Ref. Handbook of the Medical Sciences) as giving the following composition of dried bone without the removal of the marrow or blood. Water, 50 per cent; fat, 15.75 per cent; ossein, 12.40 per cent; bone earths, 21.85 per cent. Why the bone-marrow and blood should be considered and included in the determination of the organic composition of bone seems strange. These two substances are not a direct part of the bone and they should be got rid of entirely in the determination of the organic constituency. Again, these substances will give some ash and will contain some elements that are concerned in bone formation, so that the effect of calcination of fresh bone with its blood and marrow would be to add to the inorganic constituents the amount of ash of the organic parts. As a result, the percentage of inorganic would be somewhat higher than it should.

For the same reason there is no way in which the organic structure of cancellous bone can be determined, as the marrow contained cannot be got rid of in any way that would not tend to remove some of the soluble organic constituent of the bone. The percentage of organic material would be quite high in such a case.

It would seem that to get the real percentage of organic substance of compact bone, it would be necessary to remove as thoroughly as possible all traces of fat, marrow, and blood-vessels and external, or surface moisture. In that way the organic material remaining would practically belong to the bone and be a part thereof.

Primarily, the reason for the following determination was to find out, if possible, a definite relation, or variation, of the organic constituents of bone at the various ages. Just what methods of procedure were used by those who made the early determinations was not known, so some fresh bone was cleaned, weighed, calcined, and the contents determined. By this method the percentage of inherent, fixed, or component organic substance was so far above that given in the text-books that it caused surprise. There was no reason to believe that it could be an accidental variation, so that the writer immediately wondered how Berzelius, et al., made their determinations. If, according to Hoppe-Seyler, the marrow and blood-vessels were included with the bone, then we can see readily that there would be a variation, but this would make the organic content still higher than the author's determination.

In order to attempt to find out the method used by the older chemists, the determination of the organic content was carried on in a number of different ways.

So as to try to reach as near as possible the true percentage of organic substance in compact bone, five different sets of determinations were carried out. Four of these were applied to every sample of green bone and the fifth applied to the bones used for study by the students. The determinations were made on the bones of adults, varying the ages as much as possible, and stillbirths and even fetuses. In addition, in order to have a comparative anatomy relationship, determinations were also made in rabbits and cats.

The same method of preparation was used in all, except the bones used by the students for study. Certain bones only were used in all ages and in all of the animals, viz., femur, tibia, and fibula. In order to get as fair a sample of compact bone, i.e., where it would be most compact, the middle (from end to end) of each was chosen, and a section cut out and handled in a certain routine manner in all instances. The material was chosen chiefly from postmortem subjects, and not from the remains of the subjects from the dissecting-room for several reasons: 1) To avoid involving the chemicals of the embalming fluid. 2) In

order to determine the normal inherent moisture of the bone as just removed from the body. Determinations were also made in bodies that had been refrigerated for some time, and a difference was noted in the results here also.

After removal of a section of the femur, tibia, and fibula from the same sources, the flesh was allowed to remain on the others until one had been prepared for all four methods, as follows: First, the flesh of one piece of bone is all carefully cleaned off and the periosteum completely stripped, say off the femur. This caused no trouble except along the *linea aspera* of the femur and at all of the borders of the tibia and the fibula. Here the membrane adheres most tenaciously, as many processes extend into the bones at these lines and serve to anchor the membrane firmly in position. Here care must be exercised to get out all traces of Sharpey's fibers, else they might naturally add their mite to the organic constituent of compact bone, and erroneously so.

The next step is to strip out all of the marrow and then cut out all of the cancellous spicules along the inner surface of the bones. In cutting these away considerable fatty matter is thus exposed and removed, thereby getting rid of another error-producing element. When as much of this cancellous bone as possible has been removed, one can feel reasonably sure that the remainder is a real sample of compact bone. Then the bone is carefully wiped externally and internally and a section (ring) about 1 cm. in height is cut; this is then cut into quarters. Each quarter is then carefully wiped again, especially the narrow-cavity side, in order to remove all superficial moisture and fat; it is then weighed and this is the green weight. This is done with all four pieces, so that this green weight serves as an excellent check in the three other determinations.

Next the tibia is treated in the same way and then the fibula. The reason why all are not cleaned at once and then cut and weighed is because, if cleaned and left exposed to the room air, some of the moisture would escape and cause a variation in the determination. For that reason each specimen is treated in this routine manner. This may seem far-fetched, but it is of

the greatest importance, especially in the handling of fetal bones and bones of the child at birth. Here the parts must be cleaned, wiped, and weighed as rapidly as possible, or there is a marked variation, as these young bones have a greater percentage of inherent moisture. If the weighing process is slow, this variation will be noticed right on the balance pans.

By this method pieces of adult bone weighing from 1.1 grams up to over 3 grams were prepared. In fetal bones and those of the cat and rabbit, however, the weight of green bone was about 0.5 gram, as they are very bulky for their weight.

To procure and prepare four pieces of femur, tibia, and fibula and get the green weight of each requires about two hours of tedious and patient labor, but this is not the end.

The importance of the removal of all of the cancellous tissue and contained marrow will be shown in determinations A, B, C, and D. It will also show why cancellous bone proper should not be used, as the true organic constituency of osseous tissue cannot be correctly determined therefrom. This is due to the inability to get at and to remove the marrow from the cancelli.

Each of the four pieces of sample was treated as follows:

1. Green. The first piece of each bone was immediately calcined until the weight was constant and then the percentage of organic material determined, as will be shown later.

2. Oven-dried. The second piece of each bone after preliminary weighing was placed in an oven at 56°C. for twenty-four to forty-eight hours, allowed to cool, and then weighed. The loss in weight indicated the amount of moisture and the volatile organic substance present. It was then calcined until the weight was constant. By this method the percentage of moisture and volatile matter was found and also the amount of what might be termed real, or fixed organic material was obtained. In addition the green (original) weight permitted a determination of the organic material in green bone before the drying process was undertaken, giving a green-check determination.

3. Alcoholic-extracted and oven-dried. The third piece of each bone (after preliminary weighing) was placed in a 95 per cent

alcohol for twenty-four to forty-eight hours and then transferred to an oven at 56°C. for twenty-four to forty-eight hours, and then weighed when cooled. The weight lost indicated the moisture and volatile material and alcohol-soluble material. After calcining then the percentage of organic material in green bone, the percentage of moisture, volatile material and alcohol substance was next determined, and lastly the amount of remaining (fixed) organic substance.

4. Ether-extracted and oven-dried. In this determination a fresh piece from each bone (after preliminary weighing) was placed in ether from twenty-four to forty-eight hours, then oven-dried at 56°C. for twenty-four to forty-eight hours, and then weighed when cool. After calcination the percentage of organic material in green bone, the percentage of moisture, ether-soluble and volatile materials was next computed and then the amount of (fixed) organic substance remaining was computed.

The calcination was carried out by the use of two Bunsen burners. The porcelain crucible containing the green or extracted bone was placed on a triangle at an angle of about 45° and just high enough so that the light blue cone was close to the crucible. The crucible and contents were warmed gently and then the burner put in a position with some air cut off so that for the first half hour the heat would not be so intense, but sufficient to volatilize and drive off most of the carbon and volatile substances. These would ignite and burn at the mouth of the crucible. Following this, the air was turned on full to get the greatest heat and another Bunsen with all of the air turned on was held so that the blue cone was directed upon the piece of bone at the mouth of the crucible. Between these two flames the calcining was completed; the bone being turned from time to time. In this way the bone is rendered incandescent and all carbon is burned out. The time varies for different thicknesses from five minutes to half an hour. This is repeated until the weight is constant. The heat should be carefully applied at first.

In order to comprehend the following tables the method of determining these various percentages will be first given by an example:

Body 89—1920	Negro	Frozen	About 35 years of age	
	Ether—Oven-dried			
Bone No.				
Femur 90	31.6305	31.6305	4.0670 a	4.0670 a
	27.5635	27.9450	3.6845 b	2.4029 d
	4.0670 a	3.6845 b	0.3825 c	1.6641 f
	31.6305	3.6845 b	f/a = 40.90 per cent organic material in green bone.	
	29.2276	2.4029 d		
	2.4029 d	1.2816 e		
			c/a = 9.55 per cent moisture, ether-soluble substance, etc.	
			e/b = 34.78 per cent fixed organic material in the extracted bone.	
			e/a = 31.51 per cent of fixed organic material in reference to green bone.	

In all of the weighings a constant weight (50 grams) was used on the left pan and the right one contained the watch-glass, bone, and weights required to balance the 50 grams.

The various letters indicate the following:

- a = The weight of the green bone.
- b = The weight of the bone after (1) oven-drying or (2) alcohol-extracting and oven-drying or (3) ether-extracting and oven-drying.
- c = The weight of the moisture and volatile material alone or (1) + the alcohol-soluble or ether-soluble material.
- d = The weight of the calcined bone.
- e = The weight of the organic material after (1) oven-drying, or (2) after extracting and oven-drying.
- f = Organic material in green bone.
- f/a = The percentage of organic material and water, volatile and extracted material in green bone.
- c/a = The percentage of water, volatile and extractable material in green bone.
- e/b = The percentage of fixed organic material in the dried or extracted and dried bone.
- e/a = The percentage of fixed organic in the green bone.

These last two percentages exclude the water and volatile material, and while one percentage is in relation to the weight of the extracted bone, the other percentage is in relation to the weight of the green bone.

The results obtained will be tabulated with reference to age and method employed. *Fresh* indicates simple postmortem, not embalmed and not preserved in any way; *frozen* indicates storage

in the cadaver refrigerator and unembalmed; *dissection* refers to material taken from cadaver after the dissection was completed.

Green bone

Four and one-half months (fresh)

NUMBER	BONE	f/a	REMARKS
7	Fe	63.35	Fresh
8	Tib	66.05	

At birth (fresh)

1	Fe	49.61	Extremity of femur consisted mostly of cancellous bone and contained marrow and blood
2	Tib	50.44	
55	Fe	50.79	
56	Tib	53.07	
A		65.95	

20 to 60 years

84	Fem	41.15	Negro
85	Tib	41.95	47 years
84a	Fib	39.85	Frozen
112	Fem	40.61	Dissection
113	Tib	41.32	
113a	Fib	40.98	

61 to 90 years

11	Fe	40.88	71 years
15	Tib	42.46	Frozen
19	Fem	41.57	Very greasy
23	Feb	44.18	Fresh
24	Tib	44.68	Bones thin, 87 years
31	Fem	43.75	Frozen
35	Tib	45.25	
39	Fib	41.56	
43	Fe	42.28	Fresh. 76 years
44	Tib	41.40	
45	Fib	41.00	
63	Fe	48.62	80 years
64	Tib	45.77	Fresh. Bones thin and buckled in calcining
66	Fib	41.48	

Green Bone—Continued

61 to 90 years

NUMBER	BONE	f/a	REMARKS
76	Fe	39.81	Frozen. 87 years
77	Tib	41.68	
77a	Fib	40.71	
92	Fe	39.44	65 years
93	Tib	41.78	Negro. Frozen
100	Fe	43.39	Fresh. 89 years
101	Tib	39.86	
101a	Fib	40.80	

Oven-dried bone

Four and one-half months

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
9	Fe	61.23	27.41	46.59	33.82	Fresh
10	Tib	65.35	35.04	46.67	30.31	

Eight and one-half months and full term (at birth)

108	Fe	52.20	21.50	39.34	30.88	8½ months
109	Tib	53.01	22.04	39.70	30.98	Fresh
3	Fe	49.22	16.03	39.50	33.19	At birth
4	Tib	50.88	17.15	40.73	33.74	Fresh
57	Fe	52.20	19.34	40.72	33.00	At birth
58	Fib	54.95	25.52	39.55	29.46	Fresh
B		66.61	41.59	44.04	25.72	Extremity femur, mostly cancellous bone

20 to 60 years

86	Fe	40.57	8.36	35.15	32.20	Dissection
87	Tib	40.95	9.29	35.39	31.66	
87a	Fib	38.99	8.27	33.49	30.72	
114	Fe	40.28	9.22	34.21	31.06	
115	Tib	40.77	9.59	34.13	30.79	
115a	Fib	40.43	8.34	35.03	32.09	
122	Fe	42.31	7.71	37.01	33.80	
123	Tib	40.65	6.32	36.25	34.24	
123a	Fib	41.04	7.85	36.02	33.49	

Oven-dried bone—Continued

61 to 90 years

NUMBER	BONE	f/a	c/a	c/b	e/a	REMARKS
12	Fe	40.70	8.42	35.36	32.44	
16	Tib	40.48	lost	lost	lost	
16a	Fib	43.36	8.70	37.96	34.66	
25	Fe	48.63	9.86	43.51	39.65	
26	Tib	46.51	7.61	42.10	38.89	
32	Fe	42.28	3.48	40.11	38.66	
36	Tib	42.38	8.04	37.34	34.34	
40	Fib	40.00	6.85	35.58	33.14	
46	Fe	41.19	9.23	35.22	31.97	
47	Tib	42.41	11.55	34.61	30.62	
48	Fib	41.10	9.78	34.71	31.32	
67	Fe	51.50	15.16	42.52	36.10	Moisture very high, bones shrunk as they calcined
68	Tib	45.45	11.00	36.76	32.72	
69	Fib	41.85	27.76	19.49	14.82	
78	Fe	41.62	12.94	37.74	35.70	
79	Tib	39.76	7.81	34.67	31.96	
79a	Fib	39.20	5.79	35.29	33.39	
94	Fe	40.01	7.01	35.53	33.05	
95	Tib	39.22	6.80	34.77	32.41	
95a	Fib	40.09	7.82	35.15	32.48	
102	Fe	40.86	6.40	36.84	34.10	
103	Tib	41.42	6.59	37.29	34.83	
103a	Fib	41.44	7.94	36.39	33.38	

Alcohol-extracted and oven-dried bone

At birth

5	Fe	53.67	23.63	38.70	29.56	
6	Tib	53.81	30.08	39.46	27.58	
59	Fe	50.36	23.37	38.53	30.32	Extractives high. Cancellous bone in one end of sample
60	Tib	50.60	19.88	38.34	30.74	
C		68.81	44.92	43.51	23.89	Extremity of tibia. Lots of extractives and moisture

Alcohol-extracted and oven-dried bone—Continued

20 to 60 years

NUMBER	BONE	t/a	c/a	e/b	e/a	REMARKS
88	Fe	39.66	6.28	35.22	33.00	Dissection
89	Tib	39.45	6.87	35.01	32.61	
89a	Fib	38.80	8.27	33.49	30.72	
116	Fe	40.62	8.99	35.09	32.19	
117	Tib	41.44	9.60	35.73	32.77	
117a	Fib	40.09	11.17	32.69	32.42	
124	Fe	42.85	8.35	37.75	34.61	
125	Tib	41.07	7.24	35.94	33.28	
125a	Fib	41.78	9.37	35.76	32.33	

61 to 90 years

13	Fe	40.01	7.34	35.21	32.66	Eccentric results
17	Tib	43.46	9.52	37.41	33.86	
21	Fe	41.41	5.53	37.98	35.58	
27	Fe	46.55	10.54	40.20	36.04	
28	Tib	47.36	10.55	41.40	36.21	
33	Fe	40.89	7.11	36.37	33.79	
37	Tib	45.01	9.49	39.25	35.53	
41	Fib	41.24	8.46	36.31	32.50	
49	Fe	41.27	9.88	34.83	31.38	
50	Tib	42.09	11.96	24.22	30.12	
51	Fib	41.71	10.73	34.69	30.89	
70	Fe	47.42	8.37	42.45	38.90	
71	Tib	44.77	15.41	34.71	23.78	
72	Fib	43.98	11.74	36.37	32.10	
80	Fe	41.02	6.90	36.60	34.13	
81	Tib	41.28	8.47	35.84	31.77	
96	Fe	40.20	7.47	35.48	32.85	
97	Tib	40.36	7.31	35.94	33.31	
97a	Fib	40.17	6.09	36.28	33.92	
104	Fe	41.04	8.08	35.86	32.19	
105	Fib	41.11	8.34	35.75	32.77	
105a	Tib	40.42	8.00	35.24	32.42	

Ether-extracted and oven-dried bone
Eight and one-half months and full term (at birth)

NUMBER	BONE	f/a	c/a	e/b	c/a	REMARKS
110	Fe	51.35	20.04	38.95	31.14	8½ months. Fresh
111	Tib	52.44	21.53	39.38	30.73	
61	Fe	53.56	23.85	31.35	29.71	
62	Hum.	54.48	23.35	40.43	30.91	Extremity of tibia mostly cancellous
D		66.57	41.54	41.68	24.96	

20 to 60 years

90	Fe	40.66	9.55	34.78	31.51	
91	Tib	41.63	10.40	34.88	31.25	
91a	Fib	39.46	8.01	34.19	31.45	
118	Fe	41.46	9.43	33.81	30.62	
119	Tib	39.56	9.01	33.94	30.87	
119a	Fib	39.49	8.32	33.36	31.17	
126	Fe	42.59	11.58	35.08	31.01	
127	Tib	41.32	8.18	36.66	33.23	

61 to 90 years

14a	Fe	41.84	8.14	36.69	33.70	Frozen 71 years
18a	Tib	41.98	7.67	36.85	33.86	
22	Fib	40.76	6.35	36.69	34.40	
29	Fe	42.11	9.03	35.23	32.47	87 years Extractives high
30	Tib	42.48	11.29	35.17	31.18	
34	Fe	46.62	8.77	34.29	27.86	
38	Tib	39.13	8.80	33.43	30.48	
42	Fib	44.70	15.59	34.49	29.09	
52	Fe	41.42	11.01	34.19	30.41	
53	Tib	41.25	11.64	33.52	29.61	
54	Fib	41.29	10.80	34.18	30.49	
73	Fe	42.85	15.23	32.58	27.62	
74	Tib	47.89	13.57	39.70	33.56	
75	Fib	43.00	15.64	32.54	27.41	

Ether-extracted and oven-dried bone—Continued

61 to 90 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
82	Fe	43.72	13.37	35.05	30.35	
83	Tib	43.16	13.26	34.47	39.89	
83a	Fib	41.45	12.43	33.15	29.04	
98	Fe	40.60	8.17	35.37	32.50	
99	Tib	42.31	10.24	35.73	31.71	
99a	Fib	40.28	9.04	34.36	31.25	
106	Fe	41.21	10.26	34.49	30.05	
107	Tib	41.11	10.40	34.82	30.71	
107a	Fib	40.61	9.45	34.01	31.26	

Cleaned bones of study sets

NUMBER	BONE	f/a	REMARKS
160	Fe	36.73	Quite clean, dry, apparently free from grease, the tibia had a little marrow and was greasy around the marrow cavity
161	Tib	36.66	
162	Fib	37.80	
163	Fe	36.15	Fairly clean
164	Tib	35.72	Clean
165	Fib	34.41	Very clean and dry, like ivory
166	Fe	39.70	Surface greasy to the touch
167	Tib	41.54	Very greasy to the touch. Only partly degreased in the boiling
168	Fib	42.08	

Green bones

Rabbit (two-third grown)

501	Fe	35.87	
505	Tib	41.74	
509	Fe	37.00	
513	Fe	37.66	
517	Tib	41.43	

Cat (adult)

530	Fe	39.43	
531	Tib	39.69	

Oven-dried bones

Rabbit (two-thirds grown)

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
502	Fe	35.11	7.85	29.59	27.76	
506	Tib	35.08	5.67	31.15	29.41	
510	Fib	38.90	10.16	31.98	28.74	
514	Fe	40.64	12.81	31.91	27.82	
518	Tib	42.07	12.99	33.37	29.04	

Cat (adult)

532	Fe	39.10	9.75	32.37	29.31	
533	Tib	39.01	8.75	33.13	30.24	

Alcohol-extracted and oven-dried bones

Rabbit (two-thirds grown)

503	Fe	38.78	11.79	30.64	26.98	
507	Tib	38.54	10.58	31.28	27.77	
511	Fe	34.97	9.82	27.88	25.14	
515	Fe	38.38	11.54	30.57	27.04	
519	Tib	40.35	11.33	32.61	28.86	

Cat (adult)

534	Fe	39.28	10.40	32.17	28.81	
535	Tib	39.49	9.08	33.70	30.75	
535x	Tib	43.88	13.03	35.65	31.09	Extremity containing considerable cancellous bone and marrow

Ether-extracted and oven-dried bones

Rabbit (two-thirds grown)

508	Tib	41.28	12.27	33.06	28.95	
512	Tib	38.25	9.38	31.88	28.87	
516	Fe	40.24	12.18	33.54	28.08	
520	Tib	41.80	12.56	33.55	29.38	

Cat (adult)

536	Fe	39.04	9.90	32.35	29.14	
537	Tib	38.32	9.60	32.14	29.22	
537x	Tib	42.09	11.29	34.01	30.79	Extremity of shaft with cancellous bone and marrow

DISCUSSION

Green bone

By making a green weight of all pieces of bone used for these determinations, a normal green-weight percentage of the organic material is obtained in all of the different methods of after-treatment. This not only gives a greater number of results (or a better average) for this determination, but also acts as a check for the other methods. In the opinion of the writer, this green determination is the one that should be used in referring to the organic and inorganic constituents of compact bone.

Fetal bone (four and one-half months)

Upon examining the results of the determinations upon green bone, some very interesting facts are brought out. In the fetus we find that the percentage of organic substance is very high (63.99 per cent), the bone practically consists of two-thirds organic material in the fetus at four and one-half months. This leaves one-third inorganic substance. This is natural, as the bone, before its ossification, is purely organic material. In making this examination great care had to be exercised in preparing the specimen and the weighing had to be rapidly made, as the drying upon the scale pan could be noted. Again, the piece of bone free from the cancellous tissue and marrow necessarily could not be large, as the bones at that age are small.

Bones at term (eight and one-half to ten months)

At eight and one-half months and birth we note that the average is 52.15 of organic material. This is an increase in the percentage of inorganic material with a variation from 49 per cent, or practically half organic and half inorganic substances. This variation, of course, is reasonable, as the diet of the mother has a great influence upon the hardness of the bones of the child. If the mother's diet be rich in bone-forming elements, no doubt the percentage of inorganic material would be raised beyond the above figures.

In this table is the determination of bone A. Here the percentage is 65.95. Why is it so high, practically that of the fetus of the fourth to the fifth month? The note in connection with the determination states that this specimen was the epiphyseal extremity of the diaphysis (in contradistinction to the central part usually used) and that it consisted mainly of cancellous bone with its contained marrow. The percentage is practically 15 per cent above the average—a variation too great to be overlooked.

It will be remembered that at the beginning of this paper it was stated that all cancellous bone was removed before the bone was weighed, because cancellous bone contains marrow which could not be removed independently of the bone, and its presence would increase the percentage of organic material if left in the specimen. Marrow is not an organic constituent of bone and must, therefore, be removed. If it is not got rid of, then an inflated per cent of organic content is the result. The determination in the fetal bone (A, table 1) is an excellent example and proof that if a specimen contains cancellous bone, it should be entirely discarded or the cancellous bone should be completely removed. Hence the care exercised in the preparation of all specimens before weighing. This same proportionate increase will be seen in the companion determination B, C, and D.

Green bone (20 years to 61 years)

In this group the average per cent is 40.75 of organic substance. These determinations are quite close. The lowest is 38.80 per cent and the highest is 42.85, while most of the determinations are between 40 per cent and 41 per cent. In reference to these variations it might be noted that the tibia giving 38.80 per cent was from an individual 47 years of age and all other green determinations ran along this same rate, all under 40 per cent, showing that the whole series was consistent and not an individual variation due to an accidental loss. In reference to the 42.85 per cent, it might be noted that the age was 60 (border line), and again all but one were above 42 per cent, again showing a consist-

ent figure and not an abnormal variation. Now [this might be due to an unusually great amount of fat in the marrow and to unusually large haversian canals in the neighborhood of the marrow cavity, containing an unusually large amount of fatty marrow; or again, it might indicate a border-line case in which there is a slight increase of organic material in old age and in this particular individual this increase came on a little earlier than usual (an early senescence). In the next series the percentage of one case of 65 years varies between 39 and 40, and, therefore, resembles those of the younger group, indicating a comparatively youthful state.

Green bone (61 to 90 years)

In this group the ages are 65, 71, 76, 80, 87, and 89 years, giving a fairly varied series. Here we find the lowest to be 39.13 in one of 87 years. Determinations were made on the fresh subject and after the remains had been in the cold storage for quite a while; while in the fresh state, the green-bone percentages were consistently high, after freezing the percentages were consistently lower and more like 20 to 61 years of age. A variation in the moisture was also noted under these different conditions.

The highest was 51.50 and 48.03 per cent in two different individuals aged 80 and 87 years, respectively. These are isolated instances which might fairly be thrown out, but still they were retained and included in the series for the reason that this moisture and extractive content were relatively high in each case, seeming to indicate that these figures were not accidental, but in keeping with the other percentages in the experiments. In connection with most of the specimens of the individual aged 83 years, it might be mentioned that upon calcination the bones shrank, curled, or buckled and reacted like the fetal and animal bones where the organic content is relatively high or the bones are relatively thin.

Oven-dried bone

In order to try to obtain as complete a series of determination as possible, the moisture and volatile organic substances were first removed and then fixed the organic content determined. After the green weight was determined, then all of the desired specimens were subjected to the same heat (drying oven at 56°C.) to remove the moisture and ordinary volatile material (if any such were present). Then by weighing and subtracting the weight from the original green weight, the weight of the moisture and ultimately its percentage were readily determined. Then the amount or weight of the fixed organic material can be obtained. From the weight two results may be deduced: *a*) the percentage of organic substance to green bone and, *b*) the percentage of organic substance to the dried bone (green bone less its moisture). These three percentages will be taken in order.

Moisture and volatile organic material (c/a)

Four and one-half months. Here we find the percentage of moisture to average 32.43 per cent. This seems high, but we must remember that the bones are still two-thirds organic material, and organic material, in general, contains a large percentage of free water. We must also remember that the mesenchymal tissues at this stage are largely undifferentiated and so mainly embryonic in character and of a higher water content than at birth and later. We should, therefore, naturally expect a higher moisture percentage.

Eight and one-half months and full term. Here the average is 20.26 per cent with a variation from 16.03 to 25.52. By consulting the table it will be noted that the femur has a lower moisture content than the tibia, probably because the former is older from the developmental standpoint. It will also be noticed that in the eight and one-half months' fetus, the percentages are between those of the two full-term fetuses. This evidently again indicates a difference in the maternal diet. The specimen B represents the epiphyseal extremity of the diaphysis and it consisted mainly of cancellous bone and its contained marrow.

In the moisture content was 41.59 per cent due to the great quantity of blood that could not be removed. This shows why cancellous bone should not be used.

20 to 60 years. In this group while the average is 8.44 per cent, the highest is 9.59 per cent and the lowest 6.32 per cent. The highest amounts were usually noticed in the fresh (unfrozen or unembalmed bodies) and the lowest in those that had been frozen or embalmed. While there seems to be an extraction of moisture from the bones during storage in the refrigerator and an abstraction of moisture from the bones by the embalming fluid in many of the determinations, it is not constant, as will be pointed out later.

61 to 90 years. In this group there is a remarkable variation in moisture content. The average is 8.89 per cent, while the highest is 27.76 per cent and the lowest 3.48 per cent. The former occurred in the tibia of the 80-year-old individual, and this specimen curled and buckled when calcined. The lowest percentage (3.48) occurred in the 80-year-old individual after freezing, and yet other determinations of the same gave as high as 12.94 per cent. This body showed erratic results, so a number of determinations were made by various methods and all were included in the averages.

Although the average of moisture in the group 61 to 90 years is only 0.45 per cent higher than in the 20 to 60 years' group, still that little represents what the proportionate difference should be in comparing their green weights of 40.05 per cent and 42.24 per cent, respectively.

Organic material less water and volatile material in oven-dried bone

Having removed the water and volatile material, the weight of the calcined bone may now be compared with the original green weight and also with the oven-dried weight.

Relations of the organic (less moisture) to oven-dried bone (e/b)

Four and one-half months. If now we determine the percentage of organic substance after removing the water in the oven-dried bone, the average is 46.63 per cent.

Eight and one-half months to full term. In this group the average is 39.92 per cent with a variation from 39.34 per cent to 40.73 per cent—a fairly uniform series.

20 to 60 years. In this group the average is 34.52 per cent with a variation from 33.49 to 37.01 per cent. The former occurred in an individual of 35 years and the latter is one of 60 years.

61 to 90 years. In this group the average is 36.22 per cent with a variation from 19.49 per cent to 43.50 per cent. The cause of the extreme variation could not be determined and, strange to say, the average of these two extremes is nearly the general average.

Relation of organic material (less moisture) to the green weight (e/a)

Four and one-half months. In this group the average is 32.06 per cent. By adding this to the percentage of moisture, the result is 63.49 per cent of combined moisture and organic substance proper. The amount given is 63.29 per cent as per the table. All these percentages were obtained by dividing one number into the other and not by mere subtraction, hence the sum of e/a and c/a are not always equal to f/a as they would be by mere subtraction.

At eight and one-half months and full term. The average here is 31.88 per cent with a variation from 29.46 per cent to 33.74 per cent.

20 to 60 years. In this group the average is 32.23 per cent with a variation from 30.72 per cent to 34.24 per cent. The highest were those in the individual of 60 years, the lowest in those of 35 years.

Alcohol extracted and oven-dried bone

This series consists of the results of treating green bone with alcohol for twenty-four to forty-eight hours and then drying for twenty-four to forty-eight hours in an oven at 56°C. and weighing to determine the amount of moisture and alcohol-extractable substances and later the 'fixed' organic substance (in relation to the green weight and the extracted weight).

Moisture and alcohol soluble extract (c/a)

At full term. The amount of moisture and alcohol-soluble material averages 24.24 per cent in the child at birth. The lowest is 19.88 per cent and the highest 30.88 per cent. This average represents 3.98 per cent more than the mere moisture and volatile material in oven-dried bones. This does not indicate a high percentage of soluble material, indicating some little fat or oil in the compact bone proper.

20 to 60 years. In this group the average is 8.46 per cent of moisture and alcohol-soluble material. The extremes are 6.28 per cent and 11.17 per cent. The average highest is in those bones of the individual of 35 years of age, while in the subject of 60 years of age they are intermediate. The average is almost identical with the average per cent of plain moisture and volatile substance (8.44), arguing that in individual cases there may be a difference, but in the main (average) that the alcohol-soluble material is very small in quantity.

61 to 90 years. In this group the average is 9.01 per cent with variations from 5.53 per cent to 14.51 per cent. This is higher than the preceding by a half per cent and is only 0.12 per cent above the plain moisture of the oven-dried group. Evidently in the adult the compact bone contains exceedingly little alcohol-soluble substance.

Fixed organic material in alcohol-extracted and oven-dried bone
Relation of the organic substance to the extracted and
dried bone (e/b)

At full term. In this group the average is 38.76 per cent. This is only 1.16 per cent less than the same determination in plain oven-dried bone.

The average here is 29.55 per cent. This is the real organic substance in compact bone of this age. This is 1.51 per cent lower than that of the plain oven-dried bone, indicating some alcohol soluble material in the bone at birth.

20 to 60 years. In this group the average is 32.66 per cent with variations from 30.70 to 34.61 per cent. This is 0.69 per cent

lower than that of oven-dried bone, meaning that there is very little alcohol-soluble substance in compact bone.

61 to 90 years. In this group the average is 32.67 per cent with variations from 23.78 to 38.90 per cent. Compared with the corresponding determination in oven-dried bone, it is 0.59 lower than that.

Ether-extracted and air-dried bone moisture and ether-soluble substance (c/a)

At term. The average of moisture and ether-soluble substance in compact bone at birth averages 22.19 per cent with variation of 20.04 per cent to 23.85 per cent. This is 2 per cent lower than alcohol-extracted bone and about 2 per cent higher than mere oven-dried term bones.

20 to 60 years. In this group the average is 9.27 per cent with variations from 8.18 to 11.58 per cent. This average is 1.19 per cent higher than alcohol-extracted bone and 1.21 higher than oven-dried bone.

61 to 90 years. In this group the average is 10.87 per cent with variations from 8.17 to 15.60 per cent. This average represents 1.86 per cent and more than that of alcohol extracted bone and 1.98 per cent more than oven-dried bone.

Relation of fixed organic material in ether-extracted bone to ether-extracted and oven-dried bone (e/b)

At term. This average is 37.53 per cent as compared with 38.76 in the alcohol-extracted bone and 39.92 per cent in oven-dried bones.

20 to 60 years. The average in this group is 34.46 per cent as compared with 35.07 per cent in alcohol-extracted and 36.52 per cent in oven-dried bones.

61 to 90 years. Here the average is 34.83 per cent as compared with 37 per cent in alcohol-extracted, and 46.22 per cent in plain oven-dried bones.

Relation of fixed organic material in ether-extracted bone to the green weight (e/a)

At term. The average is 30.60 per cent as compared with the average of 29.55 per cent of alcohol-extracted bone and 31.88 per cent in oven-dried bones.

20 to 60 years. The average is 31.34 per cent as compared to 32.66 per cent in alcohol-extracted bones and 32.23 per cent in oven-dried bones.

61 to 90 years. The average is 30.82 per cent in this group. This is to be compared with 32.67 per cent in alcohol-extracted bones and 33.26 per cent in oven-dried bones.

Cleaned bones

In order to make the study as complete and varied as possible, it was decided to take some of the bones of the study collection sets and determine the organic content of so-called cleaned bones. For this purpose three different sets were chosen, a piece of femur, tibia, and fibula in each set to conform to the preceding tests. One set was as clean and dry as possible (A), a set that showed grease and was greasy to the touch, yet cleaned as the others had been (C), and, thirdly, an intermediate set that looked fairly clean and yet gave indication of some grease (B).

B

NUMBER	BONE	f/a	REMARKS
160	Fe	36.73	These seemed quite clean and were apparently free from grease. The tibia contained a little dried marrow and was greasy around the marrow cavity
161	Tib	36.66	
162	Fib	37.80	

A

163	Fe	36.15	The femur was fairly clean
164	Tib	35.72	The tibia was quite clean looking
165	Fib	34.41	The fibula was very clean and dry and looked like ivory

C

166	Fe	39.70	The surface of the femur was greasy to the touch.
167	Tib	41.54	The tibia and fibula were very greasy to the touch and only partly degreased in the cleaning
168	Fib	42.08	

In the test upon the fresh uncleaned bones the corresponding results are as follows:

(e/b) in oven-dried bones:	
	<i>per cent</i>
20 to 60 years	34.52
61 to 90 years	36.22
(e/b) in alcohol extracted and oven-dried bones:	
20 to 60 years	35.07
61 to 90 years	37.00
(e/b) in ether-extracted and oven-dried bones:	
20 to 60 years	34.46
61 to 90 years	34.83

In examining these results, interesting facts are noted. In group A, although the bones seemed clean, dry, and free from grease, the results varied. The tibia was the best looking and showed that all of the soluble and volatile substance had been removed and only the fixed organic material had been left. The percentage of fixed organic material in this bone was 34.41 per cent. This is practically identical with the percentage of fixed organic material in the ether-extracted bone and seems to indicate the complete extraction of all but the fixed organic substance. As we look through the other percentages we see the effect of the presence of unnoticeable and distinctly noticeable quantities of grease that remain through the incomplete cleaning of bones. Naturally, any such retained grease will have weight and will necessarily be included in percentage of fixed organic substance, though it should not be so included.

Another interesting fact is that in cleaning bones by boiling or macerating in water alone, all bones of the same body do not degrease or clean equally. Again some bones on repeated treatment seem to retain the grease to a considerable extent.

From this series it will readily be granted that the ordinarily cleaned bones are not satisfactory as a standard for the determination of organic substance in compact bone for bones that seem dry, clean, and free from grease may contain 1 or 2 per cent, and so change the final determination.

Other mammals

As some rabbits and cats were available at the time these determinations were made, it was decided to carry out the same series of tests upon the corresponding bones of these animals. The bones were prepared and treated in the same way and the tests were identical in all respects. The bones of these animals, however, are more difficult to handle. They are thin and bulky so that the amount of bone by weight was not great, varying from 0.1 gram to 0.5 gram. This did not allow a very great leeway for variations. Another prominent feature was that the bones at the first heating curled and split, and if the heat was not cautiously applied, small pieces might readily fly off and be lost. Also at the first heating the small areas of the bone that touched the crucible seemed to stick, but this did not seem to make any appreciable variation in the result.

The bones clean readily, as the periosteum strips with ease and the marrow comes out in toto as a plug would. There is no cancellous bone around the narrow cavity to be whittled away as in the human bone, so that the preparation of these bones is not so tedious a process as in the case of the human bones.

The weighing, however, must be rapidly carried out, as in the case of fetal bones, for these bones seem to contain a little higher percentage of moisture (as fetal bones) than the adult human bone. Perhaps this is only apparent and their thinness gives a greater proportionate surface for evaporation and so influences the result. There is no difficulty in getting the real green weight, however.

Rabbit. The rabbits used were all about two-thirds grown and represent the human at about the age of puberty. They were all in good health and were well developed. They had been utilized for other experimental work, but this had no influence upon the tests in hand.

Green bone. In this group the average of all of the bones tested was 38.90 per cent of organic substance, somewhat lower than we would expect to find it at the corresponding age of the human being.

The other determinations will be considered as follows:

Moisture and volatile and extractable substances

In oven-dried bone the average was 9.89 per cent.

In alcohol-extracted and oven-dried bone the average was 11 per cent.

In ether-extracted and oven-dried bone the average was 11.60 per cent.

Relations of the fixed organic substances to the extracted and dried bone (e/b)

In oven-dried bone the average was 31.60 per cent.

In alcohol-extracted and oven-dried bone the average was 30.58 per cent.

In ether-extracted and oven-dried bone the average was 33.01 per cent.

Relation of the fixed organic substance to the green bone (e/b)

In oven-dried bone the average was 28.56 per cent.

In alcohol-extracted and oven-dried bone the average was 27.16 per cent.

In ether-extracted and oven-dried bone the average was 28.82 per cent.

Cat. In the course of the laboratory work several cats were used for tissue purposes and so at the time the corresponding bones were prepared, as in the human, for these determinations. They were of adult age, well developed and apparently healthy.

Green bone. The average of all the bones tested was 38.32 per cent—somewhat low—even than the corresponding average in the human being.

Moisture, volatile, and extractable substances (c/a)

In oven-dried bone the average was 9.25 per cent.

In alcohol-extracted and oven-dried bone the average was 11.01 per cent.

In ether-extracted and oven-dried bone the average was 11.60 per cent.

*Relation of fixed organic substance to the extracted and dried bone
(e/b)*

In oven-dried bone the average was 32.75 per cent.

In alcohol-extracted and oven-dried bone the average was 33.84 per cent.

In ether-extracted and oven-dried bone the average was 32.83 per cent.

*Relation of fixed organic substance in extracted bone to green
bone (e/a)*

In oven-dried bone the average was 29.77 per cent.

In alcohol-extracted and oven-dried bone the average was 30.22 per cent.

In ether-extracted and oven-dried bone the average was 29.72 per cent.

Having compared the results obtained by the different experimental methods, it will now be of interest to consider the determinations from another standpoint, according to the following tables: Here only the adult human results and cat and rabbit will be considered.

	<i>per cent</i>
Average in green bone at 20 to 60 years	40.75
Average in green bone at 61 to 90 years	42.32
Average in green bone in rabbit	38.90
Average in green bone in cat	39.85

Moisture and extractable substance (c/a)

Average in oven-dried bone 20 to 60 years	8.44
Average in oven-dried bone 61 to 90 years	10.18
Average in oven-dried bone, rabbit	9.89
Average in oven-dried bone, cat	9.25
Average in alcohol-extracted bone, 20 to 60 years	8.46
Average in alcohol-extracted bone, 61 to 90 years	9.01
Average in alcohol-extracted bone, rabbit	11.01
Average in alcohol-extracted bone, cat	10.84
Average in ether-extracted bone, 20 to 60 years	9.27
Average in ether-extracted bone, 61 to 90 years	10.87
Average in ether-extracted bone, rabbit	11.60
Average in ether-extracted bone, cat	10.26

Relation of fixed organic substance to dried and extracted bone (e/b)

Average in oven-dried bone, 20 to 60 years	35.17
Average in oven-dried bone, 61 to 90 years	36.22
Average in oven-dried bone, rabbit	31.60
Average in oven-dried bone, cat	32.75
Average in alcohol-extracted bone, 20 to 60 years	35.07
Average in alcohol-extracted bone, 61 to 90 years	37.00
Average in alcohol-extracted bone, rabbit	30.58
Average in alcohol-extracted bone, cat	33.84
Average in ether-extracted bone, 20 to 60 years	34.16
Average in ether-extracted bone, 61 to 90 years	34.83
Average in ether-extracted bone, rabbit	33.01
Average in ether-extracted bone, cat	32.83

Relation of fixed organic substance to the green bone (e/a)

Average in oven-dried bone, 20 to 60 years	32.23
Average in oven-dried bone, 61 to 90 years	33.15
Average in oven-dried bone, rabbit	28.56
Average in oven-dried bone, cat	29.77
Average in alcohol-extracted bone, 20 to 60 years	32.66
Average in alcohol-extracted bone, 61 to 90 years	32.67
Average in alcohol-extracted bone, rabbit	27.16
Average in alcohol-extracted bone, cat	30.22
Average in ether-extracted bone, 20 to 60 years	31.34
Average in ether-extracted bone, 61 to 90 years	30.82
Average in ether-extracted bone, rabbit	28.82
Average in ether-extracted bone, cat	29.72

Green weight (f/a)

In this table the average amount of organic substance and moisture in green bone (between 20 and 60 years) is 40.75 per cent. This is 6 per cent to 7 per cent higher than that given in text-books. Of course we must admit that this includes moisture and volatile substance and perhaps a very small amount of fat or oil that could not be removed by mere physical means. The specimens were prepared as carefully as possible, and the writer feels that the bone so prepared is standard for all green-weight determinations. The question naturally arises, "Should we include moisture and volatile substances in the per cent of organic material?" Just where to draw the line seems impossible to determine. The small quantities of fat and marrow in

perimedullary haversian canals cannot be removed in this method and they are counted in as part of the organic constituent, and yet they should not be and we are helpless in any effort to remove them.

Again in comparing the results at the prime and after, we find that in the latter instance the average is 42.32 per cent—somewhat higher than in the preceding. This argues for a slightly smaller inorganic content and would argue in favor of the reduction of inorganic as being a factor in the readiness with which the bones of the old fracture made only slight strains. Although the reduction is not so great as to give positive assurance of this, still we must take it into consideration as a possibility. More determinations along this line will be made as the material presents itself.

In comparing the results in the human and in the rabbit (two-thirds grown) we note that in the latter the average percentage of organic material is 38.90 per cent. This indicates immaturity in development and would compare favorably with like stages in the human being, no doubt. Unfortunately, no material was obtainable in individuals from birth to 20 years. From the closeness in the averages, no doubt the per cents in the adult rabbit came very close to that of the human being—showing a close relation in mammals apparently.

In the adult cat the percentage was 39.85—less than 1 per cent below that of the human. If as many determinations had been made in the cat as in connection with human bone, the writer believes that the difference would have been even less, indicating a close relationship in mammals with reference to the composition of compact bones.

Moisture and volatile and extractable substance (c/a)

In this table the average moisture in compact bone in those 20 to 60 years of age is given as 8.44 per cent.

The temperature (52°C.) is not sufficiently high to cause any decomposition, nor was it employed long enough to cause any untoward effects upon the above. Whether anything else

besides moisture is driven off or not was not determined, as that was beyond the province of this paper. This amount of moisture strikes one as quite high, yet when we consider that all of our organs and tissues are principally water (mainly combined) we should not be surprised at these results. It is variable in different individuals, as the detailed results in the earlier tables show. If this be not considered normal organic content, but be subtracted from the green weight, then the average, as will be seen later, gives what is normally called 'fixed organic substance.'

Between the ages of 61 and 90 the percentage of moisture, etc., is 10.18 per cent, and as we proceed in this analysis we will note that the increase in these ages is constant. The difference between these age groups is only 1.75, yet that is a definite increase.

In the rabbit the average moisture, etc., is 9.89 per cent, and this does not seem excessive for the proportionate growth, as the percentage in the young is greater than in the adults and then in old age seems to increase again.

In the cat the amount of moisture is 9.25—nearly 0.75 per cent greater than in the adult man. This is possibly a normal condition for that animal.

In the alcohol-extracted bone the percentage is almost identical with that of oven-dried bone, being only 8.46 per cent. It seems that compact bone prepared in the way for these determinations contains very little alcohol-soluble substance. In those of 61 to 90 years the percentage is only 9.01—below the weight of ordinary moisture. This variation cannot be accounted for.

In the rabbit the average is 11.01 per cent and in the cat 10.84 per cent, showing over 1 per cent increase in both instances. Again, the relatively higher percentage in the rabbit indicates a more youthful state.

The ether-extractable substance is greater. In the human bone 20 to 60 years the average is 9.27—a material increase over moisture and alcoholic extract. This increase is constant in all. In the rabbit the average is 11.60 per cent, in the cat 10.26 per cent. In all of these forms of animals there is a higher percentage

of ether-extractable substance, and this probably is the fat in the perimedullary haversian canals. This amount of ether-extractable substance is material in relation with total amount of moisture, but in reference to the total amount of organic substance it is not so marked. We must, however, realize that there is some ether-soluble organic material even in the compact bone and something which is difficult to classify, so the question arises, "Should it be included in the organic constituent or not?"

Relation of fixed organic substance to the dried and extractable bone (e/b)

As has been seen, the percentage of organic substance in green bone includes the moisture and soluble substances, and this naturally increased that percentage. Should they be included as organic constituents or excluded? If we exclude them, then we have remaining the real fixed organic substance and then we can deduce two different answers: 1) The relation of this fixed organic substance to the weight of the extracted bone (green bone less moisture and extracted substance e/b): The relation to the original green weight (without the removal of any moisture, etc., e/a).

Of course, the percentage in reference to the extracted bone weight (e/b) will be the greater and we find that the percentage in those 20 to 60 years is 35.17 per cent. This is a per cent or so above that given in text-books, but we are not told the method of determination, and the writer for one, does not believe that this was the method used. In those of 61 to 90 years the average is 36.72 per cent—again higher than in the earlier years. In the rabbit it is 31.60 and in the cat it is 32.75 per cent—considerably lower than in the human bone.

In the alcohol-extracted bone in those 21 to 60 years, the average is 35.07 per cent, while in those 61 to 90 years, the average is 37 per cent. Again, this determination shows less alcoholic extract than mere moisture as in the last table. This seems to indicate that alcohol may fix and render insoluble or non-volatile

some substances in the bone that are volatile with mere heat or soluble in ether. So the figures would argue at any rate. In the rabbit the average is 30.58 per cent and in the cat 33.84 per cent. In the ether-extracted bone, 20 to 60 years, the average is 34.16 per cent of organic substance as compared with the extracted weight of the bone. At 61 to 90 years the average is 34.84 per cent in both instances—a drop over the corresponding moisture and alcohol-extracted percentages. This is in keeping with the relatively higher amount of ether-extractable material, and should be so. In the rabbit the average is 33.01 per cent and in the cat 32.83 per cent. These are properly proportionate results.

Relation of the fixed organic substance to the green weight (e/a)

In these results the percentages should be lower than in the preceding, as we start with a higher original weight, including therein all moisture and extractable substances. In those 20 to 60 years of age the average is 32.23 per cent and in those 61 to 90 years the average is 33.15 per cent.

In the rabbit the average is 28.56 per cent and in the cat 29.77 per cent.

These percentages could all have been got by merely subtracting the percentage of moisture from the original average in green bone, but this was not done, but each was determined from the resulting weights, so in some instances the sum of the moisture percentage and this last per cent (e/a) does not always give the exact per cent of organic substance in green bone, but the per cent according to the figures obtained. We note in all these last determinations a constant and normal difference from the preceding determination.

In the alcohol-extracted bone, 20 to 60 years, the average is 32.66 per cent and in those of 61 to 90 years the average is 32.67 per cent—almost identical. In the rabbit the average is 27.16 per cent and in the cat 30.22 per cent.

In ether-extracted bone, 20 to 60 years, the average is 31.34 per cent and from 61 to 90 the average is 30.82 per cent. In

the rabbit the average is 28.82 per cent and in the cat 29.22 per cent. These are all consistent variations and will be noted by consulting the corresponding table in the other methods of determination.

CONCLUSIONS

Five methods for the determination of organic content of bone have been here given. Of these the ordinary method of cleaning (maceration) should be avoided, as results cannot be consistent owing to the irregularity with which bones of the same individual degrease.

All of the other four methods are constant and reliable, and every one may be considered as a standard. Various points for discussion arise. Should the moisture be included in the organic content? Should any alcohol- or ether-soluble substance be included in the organic content?

If we examine the tables, we find that the only percentages close to those given in text-books are those obtained in ether-extracted bone and in relation to the weight of the bone after extraction by the ether (e/b).

This may have been the method employed by the early chemists. It was employed here to try to determine the nearest to the text-book percentages. This determination (e/b) may seem unfair because it first removes the soluble and volatile substances and takes the weight of this dried bone as a standard to determine the fixed organic substance. It does not seem fair, but it discriminates against volatile and extractable substance. It can be argued that this moisture and this soluble material are real organic contents and that they should be so included just as in making an assay of ore, the whole sample is crushed and mixed for test and not just certain parts selected. In the latter instance any desired results could be obtained and these would not be the true or fair results. If the moisture and soluble material be considered organic content, then our text-books are incorrect to the extent of 6 per cent to 7 per cent. If, on the other hand, they are excluded and the percentage still be

determined in the green weight, then the text-book percentage is 2 to 3 per cent too high. Even in oven-dried bone the percentage in reference to the dried weight is still too high—37.15 (20 to 60 years) and 36.22 (61 to 90 years).

The writer would be inclined to consider the green weight the proper one for starting. After the bone has been freed of all periosteum and cancellous tissue and surface oil, its weight should be the one used, and the ultimate results would come under the determinations f/a, in which the average is 40.75 per cent (20 to 60 years) and 42.32 per cent (61 to 90 years).

The writer would like to have the opinion of others along this line so as to establish a standard for determination of the organic content of compact bone. Further studies in this connection are under way.