LXXXIII. THE DIFFERENCE IN CHEMICAL COMPOSITION OF THE SKELETONS OF YOUNG RATS FED (1) ON DIETS DEPRIVED OF FAT-SOLUBLE VITAMINS AND (2) ON A LOW PHOSPHORUS RACHITIC DIET, COMPARED WITH THOSE OF NORMALLY NOURISHED ANIMALS OF THE SAME AGE.

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IN the course of investigations having other objects in view, much information has been accumulated relating to the histology and chemical analysis of the bones of young growing rats maintained on various defective diets. As a result it has been possible to make some new generalisations as to the chemical composition typical of normal bones and of those displaying a rachitic and osteoporotic structure respectively.

The three groups of animals whose skeletons have been thus studied may be divided into three corresponding classes according to the different diets upon which they were fed.

(1) Diets, natural or artificial, complete in all respects, including an adequate supply of the necessary minerals and of vitamins.

(2) Diet F¹, devoid of the fat-soluble vitamins A and D but adequate in other respects, which in this series of experiments usually produced osteoporosis and in a few instances slight rickets in addition.

(3) A typical rickets-producing diet, viz. McCollum's 3143 diet [McCollum and co-workers, 1921], low in phosphorus and high in calcium and providing a moderate supply of vitamin A in the large proportion of whole cereals it contains.

Among the animals receiving Diets 2 and 3 are included several which received supplements of foodstuffs calculated to correct the respective dietetic defects in varying degree.

¹ The composition of Diet F is given in the accompanying paper by Chick and Roscoe [1926].

Chemical Analysis.

The methods employed for the chemical analysis of the bones were the following.

Immediately after death the leg bones—femur, tibia and fibula—were carefully dissected, cleaned and split with a sharp scalpel in order to facilitate the subsequent extraction with alcohol and ether. The water present in the fresh bones was determined by drying for 24 hours at 105–110°. The dry bones, wrapped in silk, were extracted first with boiling alcohol for about 16 hours and afterwards with ether in a Soxhlet apparatus for 24 hours. After extraction the bones were replaced in a weighing bottle, dried, weighed and removed to a small weighed porcelain crucible to be ashed. The ashing was quickly and easily done over a Bunsen burner if the flame was provided with enough air at the later stage. The ash was heated until constant in weight, it was then dissolved in diluted hydrochloric acid, and the calcium precipitated with acid by the method of McCrudden [1909, 1911] as used by Boas [1924] and weighed as calcium sulphate. The phosphorus can be estimated in an aliquot portion of the filtrate from the calcium determination, using the method of Neumann [1902].

We have found consistently that with rats 70–90 days old the calcium content of the bone is equal to about 37 % of the total ash, irrespective of differences in the histology of the bone, corresponding to the different types of nutrition. In the present work, therefore, analysis of the ash for the proportion of calcium and phosphorus was not made in the majority of cases, but the calcium was calculated as 37 % of the weight of the ash. In Table I, however, a series of analyses is given of the ash of bones taken from young growing rats of ages varying from 54–93 days and of body weight varying from 62–161 g. All the values obtained for calcium lie between the extremes of 35.9 % and 37.2 % of the ash, with a mean value of 36.7 % for the whole series. The amount of the phosphorus present in the ash showed a greater proportional variation. With animals nourished on a low phosphorus rickets-producing diet the proportion was con-

Table I. *Analysis of the ash of the bones of young rats maintained on various diets.*

Diet	Rat No.	Age in days	Body weight g.	Ash % on fresh bone	Ash analysis		Ratio Ca/P
					Ca	P	
Complete artificial diet containing abundant butter and cod-liver oil	1	80	171	34.2	37.1	18.55	2.00
	2	80	156	35.9	37.3	17.86	2.09
				Average:	37.2	18.20	2.04
F	11	91	138	29.4	36.2	17.6	2.06
	46	92	142	27.4	35.9	18.3	1.96
	51	92	136	32.6	37.2	19.1	1.95
	53	91	161	29.2	—	—	—
	54	93	144	35.2	36.4	17.8	2.05
	197	67	54	19.7	36.7	—	—
	204	67	52	19.2	36.8	—	—
	208	68	59	24.5	36.6	18.3	1.99
	438	85	86	25.9	36.6	18.2	2.02
	440	81	82	28.3	37.0	18.1	2.04
				Average:	36.6	18.2	2.01
F + spinach	202	67	66	20.7	36.6	—	—
	210	68	68	24.1	37.0	—	—
F + milk	434	84	90	26.3	37.1	—	—
F + cod-liver oil	201	67	98	27.7	37.0	—	—
	203	67	87	27.6	37.2	—	—
				Average:	37.0	—	—
McCollum low phosphorus diet	337	58	77	14.0	36.7	16.0	2.29
	462	54	62	10.9	36.0	—	—
				Average:	36.3	—	—
McCollum + milk	331	59	92	19.2	36.8	17.8	2.07
	333	58	123	21.6	36.5	17.3	2.11
	463	54	85	17.6	37.2	—	—
	464	54	72	16.4	36.8	17.1	2.15
McCollum + cod-liver oil	357	61	66	22.9	37.0	15.3	2.41
	226	55	66	20.0	36.9	17.1	2.16
	229	55	64	22.3	37.0	—	—
				Average:	36.9	16.9	2.18

sistently lower (mean value = 16.9 % of the ash) than where the diet had contained an adequate amount of phosphates (mean value = 18.2 % of the ash).

The figure we have found for the proportion of Ca in the ash of the bones is lower than that corresponding to pure calcium phosphate $\text{Ca}_3(\text{PO}_4)_2$, viz. 38.7 % (P = 20 %) and also lower than that found experimentally by some observers. Hoppe-Seyler [1877], quoting the results of Heintz [1849], states the proportion of calcium in the ash of adult human bone to be 38.6 % and 38.5 % in the bone ash of sheep or oxen. He considered the composition to be analogous with that of apatite $\text{Ca}_{10}\text{F}_2(\text{PO}_4)_6$ with the fluorine replaced by CO_3 thus— $\text{Ca}_{10}\text{CO}_3(\text{PO}_4)_6$, a compound which contains 38.9 % of calcium. Wildt [1872] found the proportion of calcium in the ash of the bones of rabbits of ages varying from 1 day to 4 years to lie between 37.2 % and 37.7 %, while the phosphorus varied from 18.4 % to 17.4 %.

Gabriel [1894] published analyses of the bones of man, ox and goose and found great constancy in the composition of the ash in the different species. The variations were from 36.7 % to 36.4 % for calcium and from 16.0 % to 16.7 % for phosphorus. In Gabriel's method, however, the bones were not reduced to ash by heat, but the organic matter was extracted from the dried bone with alkaline glycerol at 200° and analysis made of the mineral residue or "glycerol ash." He considers that in this way it is possible to avoid the loss of a small amount of water of constitution and of carbonic acid which occurs when the bone is burnt. The proportion of calcium and phosphorus, therefore, is lower than that found present in the ash obtained by burning. In the latter, according to Gabriel, the calcium accounts for over 38 % of the ash.

Gassmann [1910], who made careful analyses of the mineral constituents in the bones of healthy and rachitic children, found 39.1 % Ca in the ash of the humerus of a healthy child 2½ years old and 37.8 % in that of a rachitic child a year younger. The phosphorus showed no change, being 17.5 % of the ash in both cases. The ratio of calcium to phosphorus was 2.23 in the healthy bone and 2.17 in the rachitic bone.

The ratio of Ca : P in the rat bones whose analyses are given in Table I is rather lower, being 2.04 and 2.01 for animals receiving respectively a complete diet and one deficient in fat-soluble vitamins, and 2.2 for those receiving a low phosphorus diet. Robison and Soames [1925] analysed a large number of the bones of rats nourished on defective diets, among them the F diet used by us, and McCollum's No. 3143 low phosphorus diet. The average value of the Ca : P ratio, was found to be 2.16 and 2.21 respectively in the bone ash of the two groups of animals. The average figure obtained by us for animals fed upon F diet was 2.01 and it is probable the difference between our results and those of Robison and Soames is due to the fact that in their experiments calcium was estimated by the method of Aron and Sebaauer [1910], while we used that of McCrudden. In Aron and Sebaauer's method the calcium is precipitated with alcohol after moist combustion of the bones with nitric and sulphuric acids and we have found that this method invariably gives a result about 3 % higher than that given by McCrudden's method, in cases where two bones of the same animal have been analysed by the two methods respectively. In McCrudden's method, when precipitating the calcium by oxalic acid after ashing the bone and dissolving the ash in hydrochloric acid, the reaction is carefully adjusted to be very faintly acid to methyl orange (p_H 4.8–5.2). This is done in order to prevent precipitation of the phosphate present, and it was conceivable that traces of calcium might remain in solution.

The method was tested by estimating the calcium present in pure Iceland spar dissolved in hydrochloric acid. In some cases sodium phosphate was added to the solution in the amount required to make the proportion of Ca to P the same as is present in bone ash. The results given below showed that the method was trustworthy.

Analysis of Iceland Spar for Calcium.

	Phosphate present	% Ca	
		found	calculated
1. Precipitation in solution faintly acid to methyl orange	+	40.32	40.00
2. " " " "	+	40.19	"
3. " " " "	0	39.95	"
4. Precipitation in alkaline solution	0	39.82	"

It is possible, nevertheless, that our calculation of the proportion of calcium in the bones as 37 % of the ash may give values which are slightly over-estimated, for, according to Gabriel, it is possible that the proportion of ash, estimated by the weight of the residue after ignition, may be slightly under-estimated, and the value of the "organic residue" slightly over-estimated. In any case the differences are slight and not significant for the purpose of this paper. The same methods were used throughout the present work, the object being to make a comparative study of the composition of bones of different types rather than to determine the absolute amounts of the various constituents present.

Histological examination.

For histological examination the rib junctions of the 6th and 7th ribs on the right side were removed, fixed with 10 % formalin in 0.85 % salt solution,

decalcified with Muller's solution and the sections stained with haematoxylin and eosin.

A diagnosis of osteoporosis uncomplicated by rickets, was made in case of bones showing (1) an abnormally thin shaft; (2) few and scanty trabeculae at the bone-cartilage junction, with an irregular arrangement in place of the usual parallel alignment; and (3) deposition of calcium in the zone of provisional calcification and in the bone tissue.

The criteria of rickets included (1) an increased and often disorganised area of proliferating cartilage; (2) an amount of osteoid tissue in excess of the normal both in the trabeculae and in the shaft of the bones, the extent of osteoid tissue relative to the amount of calcified bone, together with the degree of change in the cartilage, being regarded as a measure of the degree of rickets; (3) complete absence or defective deposition of calcium in the zone of provisional calcification. Cases in which the excess of osteoid tissue was apparent only in the shaft and trabeculae of the bones and the proliferating cartilage approached the normal in structure were diagnosed as osteomalacia.

CHEMICAL COMPOSITION CHARACTERISTIC OF THE OSTEOPOROTIC BONES FOUND IN YOUNG RATS MAINTAINED ON A DIET DEPRIVED OF FAT-SOLUBLE VITAMINS (VITAMINS A AND D).

In Table III are given complete analyses of the bones of rats 2-3 months old, killed after a period of 1-2 months on diet F deprived of fat-soluble vitamins but adequate in other respects. Here the predominant character of the histological picture was one of osteoporosis, though a slight degree of rickets was present in some instances.

Bones showing a histology typical of rickets rather than osteoporosis have also been observed in young rats nourished on diets similar to the above. This has occurred in cases where the defect in diet has been exaggerated by limiting the supply of fat-soluble vitamins to the mothers during the period of pregnancy and lactation [Korenchevsky, 1921, 1922; Korenchevsky and Carr, 1923] or when the young have received the defective diet at a very early age, when the body weight did not exceed 30 g. [Goldblatt, 1923]. In the present work the young were usually placed upon the experimental diet at about 4 weeks of age and with body weight of 40-50 g.

In Table III, section (a) contains the analyses of the bones of 12 rats which received the F diet without supplement, while the other sections of the table show the effect on the bone composition of adding various vitamin-containing foodstuffs to this diet. Vitamin A was supplied in the form of spinach (b) and milk (c), vitamins A and D simultaneously by cod-liver oil (d) and by spinach together with irradiation with ultra-violet light (e). For purposes of comparison, figures have been collected in Table II showing complete analyses of the bones of rats of similar age, which received a complete diet.

Table II. *Analysis of the bones of young rats maintained on a complete diet.*

Diet	Rat No.	Age in days	Body weight g.	Analysis of fresh bone %				Ca %		Ratio A/R	Histology
				H ₂ O	Fat	Ash	Organic residue (by difference)	On dry weight	On fat-extracted dry weight		
Natural mixed diet	3	80	136	47.0	3.4	27.2	22.4	18.4	19.7	1.21	Normal
	4	80	146	36.3	1.9	38.7	23.1	21.8	22.5	1.67	"
Complete artificial diet containing abundant butter and cod-liver oil	1	80	171	40.9	2.6	34.2	22.3	20.8	21.8	1.53	"
	2	80	156	39.8	1.7	35.9	22.6	21.5	22.1	1.59	"
Average				41.0	2.4	34.0	22.6	20.6	21.5	1.50	

Table III. *Analysis of the bones of young rats maintained on F diet (deprived of fat-soluble vitamins) without and with various vitamin-containing supplements.*

(a) Without supplement.

No. of rat	Dose daily	Litter	Age in days	Analysis of fresh bone %				Ca %		Ratio A/R	Histology
				H ₂ O	Fat	Ash	Organic residue	On dry weight	On fat-extracted dry weight		
197♂*	—	264	67	43.3	16.9	19.8	20.1	12.6	17.9	0.989	Osteoporosis and osteomalacia
204♀*	—	"	67	45.0	15.7	19.2	20.2	12.5	17.5	0.949	"
Average:				44.1	16.3	19.5	20.15	12.55	17.7	0.969	
382♀	—	218	90	42.3	13.5	24.6	19.6	15.8	20.6	1.258	Osteoporosis
387♂	—	212	89	44.2	8.3	26.5	21.0	17.6	20.7	1.265	"
427♂	—	271	86	44.2	12.1	22.4	21.4	14.5	18.5	1.058	Very slight rickets
429♀	—	"	81	44.7	13.3	21.1	20.8	13.7	18.1	1.013	Osteoporosis and? very slight r.
438♂	—	272	85	43.9	9.3	25.9	20.9	16.6	19.9	1.237	Osteoporosis and very slight r.
440♀	—	"	81	41.3	9.2	28.3	21.1	17.4	20.6	1.342	Approaching normal
443♀	—	277	72	42.5	11.4	24.9	21.2	15.6	19.4	1.175	Slight osteoporosis
448♀	—	"	78	41.7	7.4	28.9	22.0	17.8	20.4	1.310	Sl. osteoporosis. Very nearly n.
Average:				43.1	10.6	25.3	21.0	16.1	19.8	1.207	
(b) +Leaves of fresh spinach.											
200♂*	0.5 g.	264	67	46.4	14.0	19.1	20.8	12.8	17.0	0.896	Osteoporosis
202♂*	0.65 g.	"	67	48.1	9.7	20.75	21.4	14.4	17.7	0.971	Slight osteoporosis
198♂*	1.05 g.	"	67	48.4	11.2	20.51	20.5	13.9	17.7	0.970	Osteoporosis and? osteomalacia
(c) +Milk XXVIII.											
28♀	1 cc.	271	86	47.0	6.7	24.6	21.7	16.7	19.2	1.137	Very slight osteoporosis
434♂	"	272	84	41.9	10.2	26.3	21.6	16.3	19.8	1.220	Slight osteoporosis
447♀	"	277	79	39.2	8.6	29.9	22.25	17.7	20.65	1.344	Very slight osteoporosis
Average:				42.7	8.5	26.9	21.85	16.9	19.9	1.234	
426♂	2 cc.	271	86	46.1	7.0	25.4	21.5	16.9	19.5	1.180	Nearly normal
432♀	"	"	81	47.1	3.8	26.9	22.2	18.3	19.7	1.213	Very slight osteoporosis
436♀	"	272	81	41.9	8.6	28.0	21.6	17.3	20.3	1.296	"
Average:				45.0	6.5	26.7	21.75	17.5	19.8	1.230	
425♂	3 cc.	271	85	45.5	9.7	24.1	20.8	15.9	19.3	1.160	Nearly normal
433♂	"	"	87	40.2	7.55	30.3	22.0	18.2	20.85	1.378	Nearly normal. Very sl. osteop.
441♀	"	272	77	45.35	7.0	28.6	19.0	18.8	20.2	1.500	Slight osteoporosis
Average:				43.7	8.1	27.6	20.6	17.6	20.1	1.346	
431♀	5 cc.	271	82	43.7	5.6	29.1	21.7	18.6	20.6	1.341	Very sl. osteoporosis. Nearly n.
435♂	"	272	81	43.9	5.2	29.1	21.8	18.7	20.6	1.336	Nearly normal
444♂	"	277	73	45.2	3.6	28.95	22.3	19.0	20.3	1.298	"
Average:				44.3	4.8	29.05	21.9	18.8	20.5	1.325	
(d) +Cod-liver oil.											
201♂*	0.05 g.	264	67	47.9	6.9	27.7	19.6	17.7	20.4	1.303	Normal
203♀*	"	"	67	48.7	4.9	27.6	20.3	18.4	20.2	1.288	"
Average:				48.3	5.9	27.65	19.95	18.05	20.3	1.295	
(e) +Irradiated leaves of fresh spinach.											
199♂*	0.5 g.	264	67	43.7	9.5	25.5	21.3	16.3	19.6	1.197	Slight osteoporosis
205♀*	1.95 g.	"	67	44.4	5.6	28.4	21.7	18.3	20.4	1.305	"
Average:				44.05	7.55	26.95	21.5	17.3	20.0	1.251	

* Put on experimental diet at ca. 30 g. weight.

The chemical composition of the osteoporotic bone is characterised chiefly by a high proportion of fat and a low proportion of ash. These osteoporotic bones of young rats 70–90 days old contained on an average 10.6 % fat and 25.3 % ash (Table III, section (a)) while the bones from normal animals contained 2.4 % fat and 34 % ash (Table II), all figures being reckoned on the fresh weight of the bone. This excessive amount of ether-soluble material corresponds with a structure showing a slender bone shaft and a relatively large cavity filled with marrow. It is evident therefore that the composition of the bony tissue is more correctly demonstrated if the results of the chemical analysis are calculated on the fat-extracted bone. This matter is discussed more fully below.

In some instances where the young rats on F diet died before the end of the experimental period, after a period of loss of body weight caused by a reduced intake of food, the bones showed a very low proportion of fat, often less than 1 % of the fresh weight of the bones [see Chick and Roscoe, 1926, 1, rat 131, Table III]. A low fat content in the bones is usual in osteoporosis accompanied by starvation. The high proportion of fat characteristic of the osteoporosis produced by a diet deficient in fat-soluble vitamins was, however, shown when the rats maintained a fair degree of good health, with some increase in body weight and a satisfactory intake of food.

The analyses in the other sections of Table III (*a, b, c, d, e*) made upon the bones of animals which received various vitamin-containing additions to their diet, show a reduction in the fat content of the fresh bone which is in rough proportion to the vitamin value of the supplement. The figures in Table III (*c*) refer to animals which were employed for the milk tests described in detail in the accompanying paper by Chick and Roscoe [1926, 2]. In case of milk No. XXVIII, the bones of control animals (Litters 271, 272, 277) had an average fat content of 10.3 %, those of rats receiving 1 cc. milk daily still contained 8.5 % fat, while those which had received 5 cc. showed a fat content varying from 3.6 to 5.6 %.

A striking feature of the whole series of analyses contained in Tables II and III is the constancy shown in the proportion of what we have called the “organic basis” or “organic residue” of the bone, the amount of which is determined by subtracting the sum of water, fat and ash from the weight of the fresh bone. The proportion of this “organic residue,” which includes cartilage, uncalcified bone and connective tissue, amounts to from 20–22 % of the weight of the fresh bone, whether normal or osteoporotic in structure, the extremes in the average values for the whole series being 19.9 % and 22.6 %. The variations in composition of the bones corresponding to various degrees of osteoporosis are confined chiefly to the proportion of fat, of ash and, to a less degree, of water. These, together, account for 78–80 % of the total weight.

The value of the ratio of the bone ash to this organic residue, known as the A/R ratio, has been suggested [Chick and Roscoe, 1926, 1] as a “rachitic

index," or useful measure of the degree of calcification of the bones. In bones of normal rats 70–90 days old, the value of the A/R ratio was in the neighbourhood of 1.5, while a condition of osteoporosis was usually indicated by a value ranging from 0.9–1.2. In case of pronounced rickets, the value of the A/R ratio was much lower and varied from 0.4–0.8 (see below).

In much of the previous work upon this subject, the proportion of calcium contained in the dried bones has been taken as a measure of calcification, but the great variation in the fat content of the bones of young rats, when osteoporosis is produced, renders this criterion untrustworthy. The bones of rats maintained on diets deprived of fat-soluble vitamins often contain a very low percentage of calcium reckoned on the dry weight, and yet afford no evidence of rickets, when examined histologically. In such cases the low content of calcium is due rather to the high proportion of fat contained in the bones than to defective calcification of the bone tissue. For example, the average proportion of Ca in the dried bones of the 8 older animals receiving F diet alone in Table III (*a*) is only 16.1 %, while the bones of normal rats contained 20.6 %. If however the proportion of calcium be reckoned on the fat-extracted bone, the deviation from the normal is much less, viz. 19.8 % compared with 21.5 %. This is shown in an even more striking manner in case of the rats 197 and 204 (Table III (*a*)) which received the defective diet immediately after weaning, when about 20 days old. The bones of these rats after about 50 days on the experimental diet showed a calcium content in the dry bones of only 12.5 %, a figure often associated with a severe degree of rickets (see below Table IV (*b*)). They contained 16.3 % of fat and the proportion of calcium reckoned on the fat-free bone is 17.7 %. This figure is still below the normal for rats of this age (21.5 %) showing that on this diet defective calcification of the bones takes place to some extent.

CHEMICAL COMPOSITION CHARACTERISTIC OF THE RACHITIC BONES OF YOUNG RATS MAINTAINED ON A LOW PHOSPHORUS RICKETS-PRODUCING DIET.

In Table IV are set out the results of the chemical analyses made on the bones of young rats about 60 days old, which were killed after receiving McCollum's 3143 low phosphorus rickets-producing diet for 4 weeks [see Chick and Roscoe, 1926, 2]. Section (*a*) contains analyses of bones of animals which received the diet without any supplement, section (*b*) those of animals which received daily doses of butter, providing extra vitamin A and, in case of butter XXVIII, an appreciable amount of anti-rachitic vitamin D also. By the addition of milk to the diet (section (*c*)) the rats received a small amount of phosphorus in addition to both fat-soluble vitamins. Finally, in sections (*d*) and (*e*) are given analyses of bones of rats receiving respectively a daily supplement of cod-liver oil and of the unsaponifiable fraction of cod-liver oil.

Histological examination of the bones of the control animals showed an advanced degree of rickets to be present; in the animals receiving the additions of milk or butter the rachitic condition was found to be mitigated in

varying degree while those which received cod-liver oil or its products had bones showing a normal structure though not a normal composition.

Table IV. *Analysis of the bones of young rats, 54 to 68 days old, after 28 days on McCollum's 3143 low phosphorus rickets-producing diet.*

(a) Controls.

No. of rat	Daily addition to diet	Litter	Age in days	Analysis of fresh bone %				Ca %		Ratio A/R	Histology
				H ₂ O	Fat	Ash	Organic residue	On dry weight	On fat-extracted dry weight		
454 ♂	—	289	61	61.6	3.7	10.7	24.0	10.7	11.9	0.446	Severe rickets
458 ♀	—	—	61	58.15	4.1	13.0	24.7	11.9	13.2	0.524	"
462 ♀	—	302	54	59.45	6.0	10.9	23.7	9.9	11.7	0.459	"
465 ♀	—	—	54	59.5	5.0	11.8	23.65	10.5	12.3	0.499	Severe r. and osteoporosis
228 ♀	—	301	55	61.9	3.4	10.6	24.2	10.25	11.2	0.436	Severe rickets
230 ♀	—	—	56	62.45	3.3	10.6	23.7	10.2	11.4	0.446	"
Average:				60.5	4.25	11.3	24.0	10.6	12.0	0.468	

(b) Butters XXVII and XXVIII.

XXVII											
460 ♀	0.1 g.	289	61	53.3	5.0	17.6	24.2	14.5	16.2	0.727	Moderate-severe rickets
451 ♀	0.25 g.	"	61	60.4	3.6	12.5	23.5	12.2	13.4	0.535	"
459 ♀	0.25 g.	"	60	54.0	3.6	18.2	24.2	15.2	16.5	0.752	Moderate rickets
457 ♀	0.6 g.	"	64	58.2	3.6	13.8	24.4	12.7	13.9	0.566	Moderate-severe rickets
XXVIII											
452 ♂	0.1 g.	"	60	57.1	3.3	14.9	24.6	13.4	14.5	0.606	Moderate-severe rickets
453 ♀	0.25 g.	"	60	60.4	3.9	12.9	22.8	12.5	13.9	0.564	Moderate rickets
461 ♀	0.25 g.	"	68	53.0	5.6	18.2	23.1	15.0	17.0	0.789	"
455 ♂	0.6 g.	"	64	52.1	3.8	20.8	23.3	16.7	18.1	0.890	Osteoporosis and very sl. r.

(c) Milk XXVIII.

467 ♀	2 cc.	302	54	60.7	4.1	13.0	22.3	12.3	13.6	0.584	Severe rickets
464 ♀	3 cc.	"	54	58.2	3.6	16.4	21.8	14.5	15.8	0.752	Moderate rickets
468 ♀	3 cc.	"	54	58.2	3.9	15.3	22.6	13.6	15.0	0.679	Moderate-slight rickets
463 ♀	5 cc.	"	54	56.3	4.3	17.6	21.85	14.9	16.5	0.804	Slight rickets
466 ♀	5 cc.	"	54	56.7	3.4	18.3	21.55	15.7	17.0	0.679	Very slight rickets

(d) Unsaponifiable fraction of cod-liver oil. A contained vitamin D only; B contained vitamins A + D.

A											
226 ♂	0.02 g.	301	55	51.1	7.5	20.0	21.4	15.1	17.8	0.933	Normal
224 ♂	0.09 g.	"	55	49.9	4.5	23.1	22.5	17.1	18.8	1.030	Slight osteoporosis
225 ♀	0.09 g.	"	55	49.4	7.15	22.5	20.9	16.4	19.2	1.074	Normal
B											
229 ♀	0.02 g.	"	55	51.1	4.1	22.3	22.5	16.9	18.4	0.994	Normal
227 ♂	0.05 g.	"	55	49.9	5.2	22.0	22.9	16.3	18.2	0.964	"
232 ♀	0.05 g.	"	55	45.4	5.2	26.4	23.0	17.9	19.8	1.151	"

(e) Cod-liver oil.

456 ♂	0.05 g.	289	64	49.5	6.2	22.0	22.3	16.75	19.1	0.987	Nearly normal
231 ♀	0.05 g.	301	55	50.4	4.75	22.2	23.1	16.6	18.4	0.941	Normal
Average:				49.95	5.5	22.1	22.7	16.7	18.75	0.964	

It is only to be expected that bones of animals which have been deprived of phosphorus should show abnormalities arising from this circumstance. It is, therefore, proper, in an attempt to determine the abnormalities in chemical composition characteristic of rickets, to compare the rachitic bones of such rats, not with those of normal control animals but with those of animals on the same diet when rickets was prevented by administering a powerful anti-rachitic agent, such as cod-liver oil. This material appears to possess the faculty of diverting the insufficient supply of phosphorus to the bones in

preference to the soft tissues; the animal does not grow normally, but the structure of the bones approaches the normal standard.

If such a comparison be made it is clear that rachitic bone is characterised chiefly by a very high proportion of water and a very low proportion of ash. The mean value of the water content of the "severely rachitic" bones of the six control animals in Table IV (*a*) is as high as 60.5 % and of the ash content as low as 11.3 %, calculated on the fresh weight of the bones. The administration of foodstuffs containing the anti-rachitic vitamin is accompanied by a fall in the proportion of water and a large increase in the proportion of ash. With cod-liver oil the latter is doubled, the mean values obtained for the ash of the bone of animals thus treated varying from 21.9 to 23.6 %. This value still remains far short, however, of that found in the bones of normally fed animals, viz. 34 %.

The fat content of the bones of rats maintained on this diet, whether rachitic or normal as regards their structure, remained low, though slightly greater than was found in the bones of rats receiving a normal diet (Table II). The average value for six animals showing severe rickets (Table IV (*a*)) was 4.2 % of the fresh weight of the bone, while the average value obtained for bones of animals receiving cod-liver oil and showing normal histology varied from 4.8 % to 6.4 %, the figure for normal rats being 2.4 %.

The average amount of the "organic residue" present in the rachitic bones of animals fed upon McCollum's diet was a little greater than was found in the osteoporotic bones described in the preceding section but it accounted for between 1/4 and 1/5 of the fresh weight. The mean value for the control animals was found to be 24 % and this value was reduced slightly in the bones of the animals receiving cod-liver oil, where the average value varied from 22.7 % to 21.6 %. However, the total variation is small and the extreme variations in the whole series lie between 24.7 % (control rat 458) and 20.9 % (rat 225, receiving cod-liver oil).

The variation shown in the A/R ratio, on the other hand, corresponds with the histological picture. Roughly speaking, for the bones of rats fed on McCollum's diet, values under 0.5 are indicative of severe or very severe rickets [see also Protocol II in the accompanying paper by Chick and Roscoe 1926, 2], values from 0.5 to 0.7 correspond to a moderate degree of rickets, values from 0.8 to 0.9 correspond to slight rickets, while a value of 1.00 corresponds to a histological picture which approaches the normal. As was the case with osteoporotic bones, we have found this ratio the most useful indication of the degree of calcification of the bones that is yielded by chemical analysis.

In the bones of these rachitic animals, the fat content being low and the variations in this figure slight, the proportion of calcium and ash, reckoned on the dry weight of the bone, remains a useful indication of the degree of bone calcification, but in the interests of uniformity we are inclined to adopt here also the value of the A/R ratio, which in other instances is a more useful chemical criterion.

SUMMARY.

1. A condition of osteoporosis found in the bones of young rats kept on a diet deprived of fat-soluble vitamins was characterised by (1) a proportion of fat (alcohol- and ether-soluble material) in excess of the normal, (2) a proportion of ash (A) less than the normal, (3) a normal proportion of organic material, cartilage and connective tissue, etc. (R), (4) a value of the A/R ratio of about 0.9 to 1.2, the normal value being about 1.5.

2. In these osteoporotic bones the percentage of calcium, reckoned on the dry weight of the bones, is frequently very low (12 to 16 %), but is found to approach much nearer to the normal if the calculation is made on the fat-extracted bone. This value, however, is always less than normal and in spite of an absence of signs of rickets in the histological picture, the chemical analysis provides evidence of some diminution of calcification.

If the conditions are exaggerated by the use of defective diets in the breeding of the experimental animals, this diminished calcification may be associated with a histological picture recognisable as rickets.

3. The chemical composition of rachitic bones found in young rats kept on McCollum's low phosphorus rickets-producing diet was characterised by: (1) a content of mineral ash (A) much below the normal, (2) a water content much above the normal, (3) a slightly increased proportion of fat and of organic material (R), (4) a very low value of the A/R ratio, viz. from 0.4 to 0.8, as compared with 1.0, the value found for the bones of rats which received cod liver oil in addition to the low phosphorus diet.

4. If chemical examination is used in the diagnosis of rickets, the best criterion of defective calcification is given by the value of the A/R ratio or the ratio of the amount of ash to the amount of organic material contained in the fat-extracted bone.

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