Variations in the mineral composition of individual bones of the skeleton of the domestic fowl

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Before radioactive isotopes became available for the study of bone metabolism it was generally assumed that bone was a very stable tissue, although it was recognized that the detailed architecture of the bone substance was continually undergoing reorganization in particular areas. During the last 20 years or so, this view has been radically changed, mainly as a result of experiments on the uptake and release of radioisotopes by bones, and the current view is that the skeleton is in a state of dynamic equilibrium with the body fluids (Neuman & Neuman, 1958). It is recognized, however, that bones and microscopic areas within bones vary in their reactivity and therefore in the rate at which they attain equilibrium with the blood. One consequence of this relationship between blood and bone is that, provided there have been no recent large changes in the composition of the blood, all the bones of the skeleton might be expected to be very similar in chemical composition. On the assumption that it is so, it is a common practice in nutritional and physiological studies to take for analysis a single bone from an experimental animal as representative of the skeleton as a whole. During a previous investigation (Taylor & Moore, 1956) it was found that considerable differences did in fact exist between some of the bones of the skeletons of laying hens. In this paper the variation in the composition of individual bones has been further considered and the results are discussed in relation to current ideas on the relationship between bone crystals and the body fluids.

EXPERIMENTAL

Six Rhode Island Red × Light Sussex pullets were maintained in separate metabolism cages from the age of 5 months and fed on a standard laying meal containing 2% calcium, initially at the rate of 100 g daily, but increasing to 110 g as the birds entered into reproductive activity. Two of the birds were killed after 1 month and their ovaries were found to be entirely quiescent. From this time onwards, droppings were collected from the remaining birds every 2 days and analysed for calcium and phosphorus. To study the effect of pre-laying storage of Ca and P on the composition of cortical bone, it was planned to kill one bird just before calcification of the first egg-shell, thus avoiding possible changes due to withdrawal of Ca for shell formation. This bird (no. 3) was killed 7 days after a marked increase in the retention of Ca and P from the food had taken place, indicating that the secretion of oestrogen and androgen

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accompanying reproductive activity had begun (Common, Rutledge & Hale, 1948; Common, Jowsey & Maw, 1953), and at slaughter a yolk surrounded by a thin layer of albumen was present in the magnum of the oviduct. The results of the Ca and P balances for the last 13 days before slaughter are given in Table 1 which shows that the mean rates of retention of both Ca and P were more than doubled during the week before ovulation compared with the rates obtaining during the previous 6 days.

Table 1. *Weights (g) of calcium and phosphorus in the food and droppings of bird no. 3 and weights retained during the 13 days up to ovulation, showing increased rate of mineral storage from the 6th day before ovulation*

Days before ovulation	Calcium				Phosphorus			
	Food	Droppings	Balance	Retained/day	Food	Droppings	Balance	Retained/day
12–13	4·50	4·00	0·50	0·25	1·17	1·00	0·17	0·09
10–11	4·50	4·10	0·40	0·20	1·17	0·99	0·18	0·09
8–9	4·50	3·95	0·55	0·28	1·17	0·99	0·18	0·09
Mean 8–13	—	—	—	0·24	—	—	—	0·09
6–7	4·50	3·64	0·86	0·43	1·17	0·86	0·31	0·16
4–5	4·50	3·29	1·21	0·61	1·17	0·78	0·39	0·20
2–3	4·50	3·28	1·22	0·61	1·17	0·69	0·48	0·24
1	2·25	1·50	0·75	0·75	0·59	0·34	0·25	0·25
Mean 1–7	—	—	—	0·60	—	—	—	0·21

After each pullet had laid three eggs on the normal Ca ration, one (no. 4) was killed and the remaining two (nos. 5 and 6) were placed on a low-Ca diet (0·063% Ca) by withholding the calcium-carbonate supplement from the standard ration. One of these birds (no. 6) laid only one egg on the low-Ca diet and was, therefore, rejected from the experiment. The remaining bird (no. 5) was killed immediately after the sixth egg had been laid, 11 days after the low-Ca regime began.

The bones were prepared for analysis and the cortical material was analysed for Ca, P, magnesium, sodium, potassium, carbon dioxide and citrate by the methods used in previous experiments (Taylor & Moore, 1954, 1956).

Preliminary results had shown that there were considerable differences between the citric-acid contents of individual bones, and it was considered desirable, therefore, to study the variations which occur with age in the citric-acid level of pullet blood. Blood samples were taken from either the jugular or wing veins of three or four White Leghorn pullets at monthly intervals between 1 and 5 months of age, different birds being used on each occasion. In addition, blood samples were taken from four pullets shortly after they had begun to lay (between 5 and 6 months of age) and from four birds of the same age which had not yet laid, but in which the comb and wattle development associated with the onset of reproductive activity was far advanced.

Citric acid was determined on trichloroacetic-acid filtrates of the blood plasma by the method of Taylor (1953).

RESULTS

The composition of fifteen different bones from each of the five experimental birds, expressed as a percentage of the calculated ash, is given in Table 2. The bones fell naturally into two categories, one high and the other low in citrate. In general, bones

Table 2. *Composition of the bones of the pullets expressed as a percentage of calculated ash**

Bone	Calcium Bird no.†					Magnesium Bird no.†					Sodium Bird no.†					Potassium Bird no.†				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Low-citrate bones																				
Femur	37·1	37·1	37·4	37·2	36·7	0·69	0·68	0·45	0·51	0·59	0·92	0·81	0·96	1·03	1·08	0·61	0·51	0·33	0·40	0·48
Tibia	37·1	37·3	37·3	37·2	36·9	0·66	0·65	0·50	0·50	0·58	0·90	0·81	0·95	1·00	1·02	0·39	0·39	0·32	0·33	0·37
Humerus	36·9	37·1	37·0	37·1	36·9	0·53	0·65	0·52	0·49	0·69	0·90	0·84	0·98	0·92	1·01	0·33	0·32	0·29	0·34	0·33
Radius	37·2	37·2	37·2	37·0	36·9	0·63	0·60	0·53	0·45	0·58	0·89	0·87	0·97	1·04	1·04	0·47	0·48	0·32	0·47	0·38
Ulna	37·3	37·3	37·3	37·1	36·9	0·62	0·67	0·53	0·55	0·55	0·88	0·76	0·97	0·99	1·02	0·44	0·35	0·31	0·38	0·37
Metacarpals and wing digits	37·0	37·0	37·1	36·9	36·7	0·55	0·73	0·55	0·52	0·69	0·98	0·99	1·00	1·01	1·01	0·44	0·45	0·34	0·43	0·43
Metatarsus	37·3	37·3	37·2	37·1	37·0	0·61	0·64	0·47	0·50	0·56	0·83	0·87	1·13	1·08	1·13	0·30	0·32	0·29	0·25	0·33
Coracoid	37·1	37·0	37·0	36·9	36·8	0·61	0·70	0·51	0·50	0·64	0·83	0·86	0·97	1·02	0·98	0·53	0·56	0·40	0·57	0·49
Scapula	36·9	37·2	37·2	36·7	36·5	0·68	0·63	0·54	0·55	0·61	0·83	0·83	0·97	1·13	1·08	0·68	0·40	0·45	0·71	0·58
Cervical vertebrae	37·2	36·7	36·9	36·4	36·3	0·49	0·68	0·59	0·55	0·72	0·98	0·93	1·04	1·07	1·08	0·87	0·73	0·62	0·86	0·90
Mean	37·1	37·1	37·2	37·0	36·8	0·61	0·66	0·52	0·51	0·62	0·89	0·86	0·99	1·03	1·05	0·51	0·45	0·37	0·47	0·47
High-citrate bones																				
Ribs	36·5	36·9	37·1	36·8	35·9	0·65	0·67	0·55	0·56	0·78	1·01	0·94	0·91	0·98	1·16	0·78	0·52	0·63	0·85	0·97
Ilium and ischium	36·5	37·1	37·1	36·9	36·4	0·68	0·63	0·51	0·43	0·68	0·94	0·85	1·08	1·06	1·13	0·68	0·40	0·43	0·59	0·61
Sternum	36·6	37·1	37·0	36·9	36·4	0·68	0·63	0·56	0·53	0·64	0·91	0·76	0·91	0·93	1·06	0·68	0·54	0·58	0·76	0·93
Skull	36·9	36·8	37·1	36·8	36·6	0·65	0·64	0·54	0·55	0·62	0·84	0·97	0·96	1·06	1·07	0·48	0·56	0·54	0·67	0·70
Toes	36·6	37·2	37·3	37·3	36·9	0·57	0·56	0·47	0·45	0·53	1·06	1·00	0·99	1·00	1·03	0·35	0·41	0·37	0·35	0·47
Mean	36·6	37·0	37·1	36·9	36·4	0·65	0·63	0·53	0·50	0·65	0·95	0·90	0·97	1·00	1·09	0·59	0·49	0·51	0·64	0·74
General mean	36·9	37·1	37·2	37·0	36·7	0·62	0·65	0·52	0·51	0·63	0·91	0·87	0·99	1·02	1·06	0·54	0·46	0·42	0·53	0·56

Bone	Phosphorus Bird no.†					Carbon dioxide Bird no.†					Citric acid Bird no.†				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Low-citrate bones															
Femur	17·2	17·4	17·0	17·1	17·2	4·31	4·20	5·13	4·94	5·10	1·59	1·40	1·32	1·46	1·42
Tibia	17·3	17·3	17·0	17·0	17·1	4·41	4·17	5·18	4·96	5·14	1·61	1·43	1·47	1·67	1·59
Humerus	17·4	17·3	17·0	17·1	17·1	4·57	4·55	5·43	5·13	5·06	1·57	1·53	1·46	1·72	1·36
Radius	17·2	17·3	17·1	17·1	17·1	4·27	4·45	5·02	4·83	4·93	1·69	1·55	1·55	1·72	1·87
Ulna	17·3	17·4	17·1	17·1	17·2	4·38	4·26	4·93	4·94	4·90	1·41	1·38	1·36	1·55	1·55
Metacarpals and wing digits	17·3	17·3	17·2	17·2	17·1	4·34	4·23	4·89	4·69	4·99	1·83	1·60	1·51	1·77	1·61
Metatarsus	17·2	17·2	17·0	17·0	17·0	4·27	4·37	5·05	4·83	4·80	2·02	1·75	1·65	2·10	1·81
Coracoid	17·3	17·3	16·9	17·1	17·3	4·18	3·99	5·18	4·73	4·53	1·72	1·71	1·87	1·93	1·71
Scapula	17·2	17·4	17·0	17·1	17·5	4·34	4·00	4·85	4·46	4·28	1·94	1·70	1·76	2·01	1·79
Cervical vertebrae	17·2	17·4	17·2	17·4	17·6	3·79	3·71	4·37	4·11	3·81	1·90	2·09	1·76	2·00	1·70
Mean	17·3	17·3	17·0	17·1	17·2	4·29	4·19	5·00	4·76	4·75	1·73	1·61	1·57	1·79	1·64
High-citrate bones															
Ribs	17·3	17·4	16·9	17·1	17·5	3·74	3·63	4·51	4·03	3·88	2·51	2·28	2·22	2·49	1·96
Ilium and ischium	17·3	17·3	16·7	17·0	17·3	3·84	3·97	5·00	4·60	4·25	2·76	2·29	2·30	2·45	2·16
Sternum	17·2	17·1	16·9	16·8	17·4	3·93	4·12	4·52	4·41	3·73	2·71	2·47	2·62	2·87	2·30
Skull	17·3	17·3	17·2	17·1	17·2	3·94	3·58	4·10	3·92	3·97	2·36	2·58	2·21	2·68	2·54
Toes	17·3	17·1	16·8	16·8	16·8	4·25	4·13	4·70	4·54	4·82	2·49	2·30	2·37	2·72	2·60
Mean	17·3	17·2	16·9	17·0	17·2	3·94	3·89	4·57	4·30	4·13	2·57	2·38	2·34	2·64	2·31
General mean	17·3	17·3	17·0	17·1	17·2	4·17	4·09	4·86	4·61	4·54	2·01	1·87	1·83	2·07	1·86

* Calculated by the method used by Taylor & Moore (1956).

† Birds nos. 1 and 2, before pre-laying storage had begun; bird no. 3, after pre-laying storage but before laying; bird no. 4, after laying three eggs on normal-Ca diet; bird no. 5, after laying six eggs on low-Ca diet.

in the former category (ribs, ilium and ischium, sternum, skull and toes) contained more than 2% citric acid in the ash (calculated by the method used by Taylor & Moore (1956)), and those in the latter (the wing and leg bones, coracoid, scapula and cervical vertebrae) less than 2%. An inverse relationship between the citrate and carbon-dioxide contents of the ndividual bones was clearly demonstrated when correlation coefficients between the percentages of these two constituents in all fifteen bones were calculated for each bird:

Bird no.	1	2	3	4	5
r	−0·73	−0·79	−0·61	−0·70	−0·53
Level of significance	1%	0·1%	5%	1%	5%

When the Ca, P, Mg and Na contents of individual bones were considered, no characteristic features of particular bones were apparent and the small differences between bones of the same skeleton did not in general appear to be consistent from bird to bird. The toes, however, tended to be higher in Ca and lower in Mg than the general mean.

There were considerable differences between the K contents of individual bones. The tibia, humerus, radius, ulna, metacarpals and wing digits, metatarsus and toes, were generally lower in K than the mean, whereas the cervical vertebrae, ribs, sternum and skull were higher. There was a marked tendency for the bones rich in K to be rich also in citric acid, but the toes were exceptional in this respect.

The 'labile' bones (as defined by Taylor & Moore, 1956) of bird no. 5 which had laid six eggs on the low-Ca diet, when compared with a laying bird that had not been subjected to a low-Ca treatment, showed changes similar to those reported previously (Taylor & Moore, 1956), i.e. a decrease in Ca and CO_2 and an increase in Mg, Na, K and P.

The bones of bird no. 4, which had laid three eggs on the high-Ca diet, were very similar in composition to those of bird no. 3 which was killed immediately after her first ovulation, and the most interesting comparisons were between the bones of these birds and of the two immature pullets, i.e. between birds in which reproductive activity, with its concomitant blood changes, had been initiated and birds that had not entered into reproductive activity.

The mean composition of the ash of the high- and low-citrate groups of bones obtained from these two pairs of birds is given in Table 3. Compared with those of the immature pullets the bones of the sexually mature birds were higher in CO_2 and Na and lower in P and Mg. The differences between the mean percentage of each of these constituents in the individual bones of the mature and immature pullets were compared statistically by the t test and these differences were all highly significant ($P < 0{\cdot}001$). Ca, K and citrate showed no consistent changes due to the onset of reproductive activity.

The amounts of citric acid present in the plasma of the pullets of different ages are given in Table 4. There were considerable variations between birds but before puberty there was no consistent trend associated with increase in age. The mean citric-acid content of the plasma of the seventeen pullets from 1 to 5 months of age was 8·3 mg/100 ml, compared with the 2–3 mg/100 ml normally found in human blood (Harrison

& Harrison, 1952). The laying birds showed very little variation in their plasma citric acid, the mean value being 2·8 mg/100 ml, and the birds of the same age which had not laid but which showed comb and wattle development had a mean value of 4·3 mg/100 ml. There was, therefore a marked reduction in the citric acid of the blood of pullets associated with reproductive activity.

Table 3. *Mean percentage composition of the ash of the bones from the immature pullets (nos. 1 and 2) and from the pullets that had entered into reproductive activity (nos. 3 and 4)*

	Calcium Birds nos.		Magnesium Birds nos.		Sodium Birds nos.		Potassium Birds nos.		Phosphorus Birds nos.		Carbon dioxide Birds nos.		Citric acid Birds nos.	
Bones	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4	1, 2	3, 4
Low-citrate	37·1	37·1	0·64	0·52	0·88	1·01	0·48	0·42	17·3	17·1	4·24	4·88	1·67	1·68
High-citrate	36·8	37·0	0·64	0·52	0·93	0·99	0·54	0·58	17·3	17·0	3·92	4·44	2·40	2·49
General mean	37·0	37·1	0·64	0·52	0·89	1·00	0·50	0·47	17·3	17·1	4·13	4·74	1·94	1·95

Table 4. *Citric-acid content (mg/100 ml) of the plasma of White Leghorn pullets of different ages. The birds came into lay at different times over a period of about 2 weeks between 5 and 6 months of age*

(Values for individual birds)

Age (months)							
						5–6	
						Non-laying*	Laying
1	2	3	4	5			
7·44	11·16	8·09	7·60	7·90		3·72	2·86
8·08	9·40	8·25	6·80	8·34		4·76	2·88
8·37	7·31	9·85	7·04	8·93		4·32	2·86
—	—	—	7·68	7·77		4·37	2·72
Mean 7·96	9·29	8·73	7·28	8·24		4·29	2·83

* The comb and wattle development associated with reproductive activity was far advanced in these birds.

DISCUSSION

It is clear that the largest variations in the composition of the mineral fraction of individual bones of the skeletons of pullets occur in their citrate and CO_2 contents. In bird no. 1, for example, the citrate content of the ash varied from 1·4 to 2·8%, and that of CO_2 from 3·7 to 4·6%. It is probable that both of these constituents occur externally to the apatite lattice of the bone crystals (Hendricks & Hill, 1950; Neuman & Neuman, 1953; Taylor, 1955, 1959) and, since there is a rough inverse relationship between them, it seems reasonable to suggest that both CO_3^{2-} and Cit^{3-} ions are adsorbed at the same sites on the apatite crystals, probably on the surface Ca^{2+} ions. Largely as a result of the work of Sobel, Rockenmacher & Kramer (1945) it is now generally accepted that the composition of bone minerals reflects the ionic composition of the blood at the time the crystals are formed, though they may undergo changes subsequently in response to changes in their ionic environment, and it is difficult to avoid the conclusion that the variations in the citrate:CO_2 ratio of the different bones

must be due to variations in the citrate:CO_2 ratio of the tissue fluid bathing them during their calcification. Neuman, Firschein, Chen, Mulryan & DiStefano (1956) have shown with dogs that local variations occur in the blood-citrate level after injections of parathyroid hormone, and Martensson (1938) observed that the citric-acid content of the portal blood of rabbits and cats was higher than that of the arterial blood.

Buffa & Peters (1949–50) showed that there were considerable differences in the citric-acid content of the various soft tissues in the pigeon and that the brain had a higher concentration of citric acid than any of the other tissues studied. We have made similar observations on the hen and it is possible, therefore, that the blood supply to the skull is higher in citric acid than the general circulation, which may explain the high citric-acid content of the skull. The CO_2 content of blood may also show local variations depending largely on the metabolic activity of the tissues from which the blood is draining. Until detailed work is done on the variations in the citric-acid and CO_2 content of venous blood from different regions, this suggested explanation for the variations in the citrate and CO_2 content of individual bones must remain purely tentative.

Though the importance of local and general variations in the composition of the blood in influencing the composition of the bone salts must be recognized, the role of the bone cells must not be overlooked. The composition of the tissue fluid in the zones of calcification must be affected by the metabolic activities of the bone cells, and variations in their production of citric acid may well be a major factor in determining the amounts of citrate and CO_2 present in different bones. Increased local production of any acid would be expected to result in a reduction in the CO_2 content of the bone salts in the immediate vicinity, since it is known that in conditions of acidosis there is a general reduction in the CO_2 content of the skeleton (Marek, Wellmann & Urbanyi, 1934).

It has been shown above that the mean citric-acid level of the venous blood of pullets from 1 to 5 months of age, the period during which the bones of the experimental birds would have been calcified, is 8·3 mg/100 ml, and that the mean level falls to approximately 4·3 mg/100 ml with the onset of reproductive activity and to 2·8 mg/100 ml when laying begins. This reduction is probably associated with oestrogen secretion (Common, Bolton & Rutledge, 1946–8). Common (1941) observed that the alkali reserve of pullets given a high-Ca ration similar to that used in our experiments increased from approximately 60 vol. % CO_2 to 80 vol. % during the pre-laying period so that the CO_2:citrate ratio of the blood must increase greatly at this time. It is during this period that the medullary bone is laid down and it is relevant to note that the CO_2:citrate ratio of the medullary bone of birds given a high-Ca ration is even greater than that of the low-citrate bones (Taylor & Moore, 1956). The cortical tissue of all the bones of the skeletons of the sexually mature birds showed an increase in CO_2 compared with that of the immature pullets, reflecting the increase in the blood alkali reserve, but their content of citrate remained unchanged, in spite of the large reduction in the blood citric acid. Harrison, Harrison & Park (1958) observed that the bone citrate of rats increased in parallel with a rise in serum citrate and remained high when the concentration of citrate in the serum fell again.

Compared with the bones of the immature pullets, there was a mean increase of 0·61% CO_2 and 0·12% Na and a mean decrease of 0·26% P and 0·12% Mg in the bones of the mature birds (Table 4). In terms of equivalents, the mean increase of CO_2 (0·61% CO_2 = 27·7 m-equiv. CO_3^{2-}) was similar to the mean decrease in P (0·26% P = 25·2 m-equiv. PO_4^{3-}) and it seems reasonable to suggest that carbonate ions replaced adsorbed phosphate ions on the apatite crystals as the alkali reserve increased. Neuman (1953) has shown that this heteroionic exchange process takes place when synthetic hydroxyapatites are exposed to solutions containing increasing amounts of CO_2 and that the anionic composition of the crystal surfaces depends on the ratio of carbon dioxide to phosphate in the liquid phase.

Since phosphate rather than citrate ions were replaced, it would appear that the latter are more firmly adsorbed on to the bone crystal than phosphate, which in view of the stability of the $(CaCit)^-$ ion is not perhaps surprising. The strength with which the citrate is adsorbed may well explain the fact that the high-citrate bones retain their citrate in the face of the falling blood-citrate levels associated with the onset of reproductive activity.

Heller & Pursell (1937) have found no change in the plasma Na associated with reproductive activity in fowls. We have confirmed this finding and have also observed that the total plasma Mg increases from a mean level of 2·1 mg/100 ml in immature birds to 3·5 mg/100 ml in laying pullets, the corresponding diffusible Mg levels being 1·8 and 2·1 mg/100 ml, respectively (unpublished observations).

It is clear that the increase in Na and the decrease in Mg observed in the bones of the pullets as they became sexually mature cannot be explained in terms of changes in the plasma concentration of these elements. As a result of in vitro experiments on the solubility of Mg in bone minerals, Taylor (1955) has shown that the Mg occurs in two forms, one relatively soluble and the other relatively insoluble, and suggested that the former fraction might be adsorbed by secondary electrostatic attraction on to adsorbed phosphate ions. If this hypothesis is correct, it would afford an explanation for the simultaneous decrease in Mg and phosphate observed in the bones of the mature pullets. Not enough is known about the spatial relationships between the surface ions of the apatite lattice and the ions adsorbed on the crystal surfaces to enable a precise explanation to be given for the increase in Na which occurred in the bones of the mature pullets compared with the bones of the immature birds, but if adsorbed phosphate carrying magnesium ions were, in fact, displaced by carbonate during the period immediately before the laying of the first egg, there would be a net negative charge induced on the crystal surfaces and, since Na^+ is the cation which predominates in the tissue fluid, uptake of this ion would be expected.

It has been shown above that the Mg, Na, phosphate and CO_2 contents of hen bones are readily influenced by changes in the composition of the blood and it may reasonably be assumed, therefore, that these constituents of bone are in equilibrium with the blood. The considerable differences between the amounts of K present in different bones of the same skeleton suggest that the K of bone may not be in equilibrium with that of the blood, but a more probable explanation is that the different bones were contaminated by bone marrow to varying degrees. Extreme care was

taken to eliminate this contamination by fine grinding (300 B.S. mesh) and subsequent purification by a flotation technique with carbon tetrachloride, but any marrow within individual bone particles would not be removed by this method. With the exception of the skull, all the bones that were found to be rich in K are well supplied with marrow. Evidence that the K of bone does respond to changes in the ionic composition of the blood is the large and rapid increase in the bone K which occurs when laying hens are placed on a low-Ca diet (Taylor & Moore, 1956). No information concerning the relationship between bone and blood Ca is available from this experiment, but there is abundant evidence from other sources that blood Ca is in equilibrium with the exchangeable fraction of the bone Ca (Neuman & Neuman, 1953).

It seems probable, therefore, that of the various bone constituents considered here citrate is the only one that is not in equilibrium with the blood. It appears that citrate, phosphate and carbonate ions compete with one another for the anion-adsorbing sites on the apatite crystals during their formation and that the proportions in which these ions occupy the available sites depend on their relative concentrations in the tissue fluid in the immediate neighbourhood of the growing bone crystals. Whereas the adsorbed carbonate and phosphate ions are in equilibrium with the tissue fluid, changing in amounts with changes in the $P:CO_2$ ratio of the plasma, the citrate ions appear to be so firmly adsorbed that they are not influenced by changes in the blood-citrate levels. Armstrong & Singer (1956) have reported, however, that citrate can be displaced from bone powder in vitro by very high concentrations of phosphate and that high concentrations of citrate (30 mg/100 ml) displace CO_2.

If the results of this experiment prove to be applicable to all species of experimental animal, it would appear to be legitimate to take a single, well-chosen bone for analysis in nutritional studies in which Ca, P, Mg or Na are being investigated, but that the variations in the K, CO_2 and citrate content of different bones of the same skeleton may be so great that this procedure could lead to serious errors when these mineral constituents are of primary interest.

SUMMARY

1. Fifteen bones of the skeletons of five pullets have been analysed for calcium, phosphorus, magnesium, sodium, potassium, carbon dioxide and citrate. Two of the birds were sexually immature, one had laid three eggs on a normal-Ca diet, one had laid six eggs on a low-Ca diet and one was killed shortly after ovulation of the first ovum and before shell calcification had begun.

2. The bones fell into two categories on the basis of their citric-acid content, one containing more than 2% citric acid in the calculated ash (ribs, sternum, skull, toes, ilium and ischium) and the other containing less than 2% (wing and leg bones, coracoid, scapula and cervical vertebrae).

3. There was an inverse relationship between the citric-acid and the CO_2 content of the bones.

4. No consistent differences between the Ca, P, Mg and Na contents of individual bones were observed, but, with the exception of the toes, the high-citrate bones tended to be richer in K than the low-citrate bones.

5. The onset of reproductive activity was associated with increases in the Na and CO_2 and decreases in the Mg and P contents of the skeleton, but no changes were observed in the bone citric acid.

6. The mean plasma content of citric acid fell from 8·3 mg/100 ml to 2·8 mg/100 ml when ovarian activity began.

7. It is suggested that the changes in bone composition associated with the onset of reproductive activity were due primarily to the decrease in the $P:CO_2$ ratio of the blood which occurs at this time, and that all the constituent ions of bone minerals investigated are in equilibrium with the blood with the exception of the citrate.

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