30. STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM IN RELATION TO THE CHEMICAL STRUCTURE OF BONE

1. EXPERIMENTS WITH LAYING BIRDS

By CYRIL TYLER

From the Royal Agricultural College, Cirencester

(Received 6 November 1939)

In a recent paper Common [1938] has suggested that the skeleton of a laying bird undergoes modifications in composition due to alterations in the ratio of carbonate CaO to total CaO and in the ratio of residual CaO to total CaO. He defines residual CaO as that CaO not present in the form of $\text{Ca}_3(\text{PO}_4)_2$. Brook *et al.* [1934] have stated that it is possible to change the ratio of $\text{Ca}_3(\text{PO}_4)_2$ to CaCO_3 in the bones of rats by dietetic means, whilst Burns & Henderson [1936] have shown that, within limits, the ratio of carbonate to total Ca in bone appears to be a measure of its age. Further, Marek *et al.* [1937] report that feeding CaCO_3 to young pigs increased the Ca : P ratio of the bones.

Thus there appears to be some evidence that the composition of bone can alter, which in turn suggests that it is possible for bone to gain or lose Ca without gaining or losing an exactly equivalent amount of P. If this be admitted, then the possibility arises that Ca may be gained or lost from the bone without any change in its P content and *vice versa*. Such conceptions are contrary to the generally accepted idea that the composition of the skeleton is fairly constant and contrary to the evidence of many experiments which indicate concomitant losses or gains of Ca and P. It was therefore decided to attempt to obtain further information on this subject.

Experiments carried out by the author [Tyler, 1935] with a view to comparing the relative efficiencies of various Ca compounds in egg production yielded results which on re-examination furnish some interesting data concerning changes in the chemical composition of the bone during egg laying.

In the experiments referred to, two Rhode Island Red pullets (or hens) were housed in separate metabolism cages and fed on weighed amounts of a basal ration of commonly used poultry foods, which was low in Ca but adequate in every other respect. Distilled water was constantly available for drinking purposes and the basal ration was supplemented in the first experiment with calcium carbonate, in the second with calcium sulphate and in the third with calcium gluconate, the supplement providing equal quantities of Ca in each case. At the same hour of each day food residues, faeces and eggs were collected from each bird separately and fresh food and water were given. Food, food residues, faeces, egg shell and egg content were analysed for Ca and P and daily balances were struck. Each experiment lasted 35 days and was preceded by a preliminary period of about 5 days on the same diet. Different pairs of birds were used for each experiment and the three experiments were not carried out concurrently.

In the 1st and 3rd experiments one of the two birds moulted so that the results are only available for four laying birds. It is hoped to consider the results for the two moulting birds in a later paper.

METHODS OF ANALYSIS

Ca was estimated by precipitating it as oxalate and titrating with standard potassium permanganate. P was estimated by precipitating it as ammonium phosphomolybdate and titrating with standard alkali, using the method of Pemberton & Neumann as modified by Richards & Godden [1924].

RESULTS

For ease of reference the birds have been designated C2, S1, S2 and G2, indicating respectively the bird receiving calcium carbonate, the two birds receiving calcium sulphate and the bird receiving calcium gluconate.

Table 1 gives the relevant data regarding these four birds.

Table 1

Bird	No. of eggs laid	% Ca in food	% P in food	Ca : P of food	Ca balance over period g.	P balance over period g.	Ca : P of balance
C2	18	1.68	0.40	4.20	-3.11*	-1.57*	1.98
S1	17	1.76	0.37	4.76	-5.83	-0.49	11.90
		1.76	0.43	4.09			
S2	21	1.76	0.37	4.76	-8.11†	-0.67†	12.10
		1.76	0.43	4.09			
G2	18	1.65	0.32	5.16	+4.55*	-1.60*	—

Note. The two sets of Ca and P values for the food given in the case of birds S1 and S2 indicate that these birds received the same ration but during the last 14 days of the experimental period the P content was slightly higher than during the first 21 days. This was due to the use of a fresh consignment of feeding stuffs after the first 21 days and not to any radical change in the nature of the ration.

* 34 day balance }
 † 32 day balance } due to breakage of eggs on other days.

If the values for the balances of Ca and P shown in Table 1 represent the changes in bone Ca and P then obviously there must be some alteration in the composition of the bones since the Ca/P ratio of bone is 2.2 : 1 and in only one case, namely C2, is the Ca/P ratio of the balance anywhere near 2.2 : 1; and further, in the case of G2 a positive Ca balance is associated with a negative P balance.

It is felt that as long as the birds are neither gaining nor losing flesh rapidly (and these birds were fairly constant in weight throughout the period) then the total Ca and P content of the tissues other than bone will remain at least sufficiently constant so as not to affect calculations regarding the relatively large quantities of Ca and P taking part in bone changes during a laying period. It is only when tissue other than bone is being rapidly broken down or built up that such calculations are likely to be seriously upset.

In further support of this contention are the values obtained if it is alternatively assumed that bone breaks down or builds up on the basis of a Ca:P ratio of 2.2 : 1. In such a case the values obtained for Ca or P stored in or lost from tissues other than bone are far in excess of changes which the tissues could stand. In the case of bird G2, for example, if the Ca:P ratio of bone changes is 2.2 : 1 then:

(a) assuming that no Ca changes take place in the tissues, the balance of Ca will represent the bone changes of Ca and from this a simple calculation shows that the P lost from the tissues would be 3.67 g.

or (b) assuming that no P changes take place in the tissues, the balance of P will represent the bone changes of P, and from this a simple calculation shows that the Ca gained by the tissues would be 8.07 g.

Such values represent the two extremes of change but calculation of intermediate values reveals changes equally outside the range of physiological probability.

Similarly improbable figures can be obtained from the data for birds S1 and S2.

Thus the assumption appears justified that the values for the Ca and P balances in these cases represent, in the main, changes in the bone Ca and P, and that the bones of these birds must therefore be changing in composition.

It was felt that little further information could be obtained from total balances over the whole experiment and that some evidence was needed as to what part of the bone compound was changing. With this in mind the following calculation, similar to that used by Common [1938], was made for each day of each experiment.

The P balance figure was multiplied by the factor 1.93 (Ca: P ratio of $\text{Ca}_3(\text{PO}_4)_2 = 1.93:1$) to give the value of the Ca lost or gained by the $\text{Ca}_3(\text{PO}_4)_2$ portion of the bone. Then, by subtracting this figure from that for the total Ca

Table 2. *Bird C2. Ca_P and Ca_R lost or gained by bone, the retention of Ca (Ca_A) and egg shell Ca (Ca_S), (g.)*

Day	Laying days				Non-laying days		
	Ca_P	Ca_R	Ca_A	Ca_S	Ca_P	Ca_R	Ca_A
1	—	—	—	—	-0.39	+1.55	+1.16
2	-0.95	-0.29	+0.83	2.03	—	—	—
3	-0.69	+0.09	+1.41	1.98	—	—	—
4	—	—	—	—	+0.46	+0.75	+1.21
5	-0.23	-0.62	+1.01	1.82	—	—	—
6	Omitted because of incomplete data				—	—	—
7	—	—	—	—	-0.81	+2.01	+1.20
8	+0.37	-1.78	+0.68	2.04	—	—	—
9	+0.04	-0.68	+1.27	1.85	—	—	—
10	—	—	—	—	-1.20	+2.34	+1.14
11	+0.33	-1.41	+1.00	2.04	—	—	—
12	-0.35	-0.87	+0.70	1.88	—	—	—
13	—	—	—	—	-0.31	+1.58	+1.27
14	+0.31	-1.23	+1.16	2.03	—	—	—
15	-0.41	-0.40	+1.22	1.99	—	—	—
16	—	—	—	—	+0.69	+0.43	+1.12
17	-0.66	-1.27	+0.00	1.87	—	—	—
18	—	—	—	—	+0.44	-0.01	+0.43
19	—	—	—	—	+0.60	+0.17	+0.77
20	—	—	—	—	+0.41	+0.24	+0.65
21	-0.62	-0.53	+0.74	1.84	—	—	—
22	—	—	—	—	+0.58	-0.02	+0.56
23	—	—	—	—	+0.58	+0.22	+0.80
24	—	—	—	—	+0.37	+0.39	+0.76
25	-0.60	-0.72	+0.68	1.94	—	—	—
26	—	—	—	—	+0.48	+0.36	+0.84
27	—	—	—	—	-0.31	+1.26	+0.95
28	+0.35	-1.32	+1.00	1.91	—	—	—
29	-0.75	-0.29	+1.04	2.03	—	—	—
30	—	—	—	—	+0.44	+0.24	+0.68
31	-0.41	-0.58	+1.10	2.04	—	—	—
32	—	—	—	—	-0.50	+1.42	+0.92
33	+0.21	-1.32	+1.03	2.08	—	—	—
34	—	—	—	—	-0.41	+1.27	+0.86
35	-0.10	-1.04	+0.89	1.99	—	—	—
Sum	-4.16	-14.26	+15.76	33.35	+1.12	+14.20	+15.32
Mean	-0.24	-0.84	+0.93	1.96	+0.07	+0.84	+0.90

balance the value of the residual Ca lost or gained by the bones was obtained. Tables 2, 3, 4 and 5 show the values obtained by this calculation and in addition the amounts of Ca in the egg shells and the amounts of Ca retained on any one day. This latter figure is not a measure of actual absorption but is simply the difference between the intake of food Ca and the output of "faecal" Ca. It must also be remembered that "faecal" Ca or any other constituent of the "faeces" represents, in the case of birds, both urinary and faecal excretion.

There has been, and still is, some controversy about the chemical structure of bone. Various formulae have been advanced such as $\text{CaCO}_3 \cdot n\text{Ca}_3(\text{PO}_4)_2$ where $n=2$ [Bogert & Hastings, 1931] $\text{Ca}((\text{Ca}_3(\text{PO}_4)_2)_6) (\text{OH})_2$ [Morgulis, 1931;

Gassmann, 1931]. But if, when discussing the chemical composition of bone, the terms "Ca of $\text{Ca}_3(\text{PO}_4)_2$ " and "residual Ca" are used the controversy is avoided and has no influence upon the results. These two terms can with convenience be abbreviated to Ca_P and Ca_R respectively and such abbreviations will be used in this paper.

Table 3. *Bird S1. Ca_P and Ca_R lost or gained by bone, the retention of Ca (Ca_A) and egg shell Ca (Ca_S), (g.)*

Day	Laying days				Non-laying days		
	Ca_P	Ca_R	Ca_A	Ca_S	Ca_P	Ca_R	Ca_A
1	—	—	—	—	+0.17	+0.30	+0.47
2	-0.54	-0.41	+0.57	1.46	—	—	—
3	—	—	—	—	+0.39	+0.25	+0.64
4	—	—	—	—	+0.42	+0.05	+0.47
5	—	—	—	—	+0.29	-0.08	+0.21
6	—	—	—	—	+0.10	+0.45	+0.55
7	—	—	—	—	+0.41	-0.07	+0.34
8	-0.66	-0.45	+0.62	1.68	—	—	—
9	—	—	—	—	+0.29	+0.48	+0.77
10	-1.00	-0.71	+0.15	1.80	—	—	—
11	—	—	—	—	+0.31	+0.18	+0.49
12	—	—	—	—	+0.41	+0.25	+0.66
13	-0.79	-0.81	+0.31	1.86	—	—	—
14	—	—	—	—	-0.04	+0.97	+0.93
15	-0.17	-1.30	+0.44	1.87	—	—	—
16	-0.64	-0.48	+0.80	1.88	—	—	—
17	—	—	—	—	-0.52	+1.18	+0.66
18	+0.27	-1.20	+0.79	1.66	—	—	—
19	-0.58	-0.36	+0.85	1.74	—	—	—
20	—	—	—	—	-0.10	+1.16	+1.06
21	-0.19	-0.67	+0.79	1.59	—	—	—
22	+0.14	-1.03	+0.66	1.49	—	—	—
23	-0.37	-0.69	+0.54	1.55	—	—	—
24	—	—	—	—	+0.02	+0.75	+0.77
25	+0.39	-0.92	+0.81	1.29	—	—	—
26	-0.21	-0.40	+0.93	1.48	—	—	—
27	—	—	—	—	-0.19	+1.21	+1.02
28	+0.25	-0.87	+0.88	1.45	—	—	—
29	—	—	—	—	+0.56	-0.21	+0.35
30	—	—	—	—	+0.10	+0.70	+0.80
31	+0.15	-1.14	+0.57	1.51	—	—	—
32	-0.17	-0.68	+0.72	1.52	—	—	—
33	—	—	—	—	-0.27	+0.90	+0.63
34	+0.39	-1.09	+0.74	1.39	—	—	—
35	—	—	—	—	+0.50	-0.21	+0.29
Sum	-3.73	-13.21	+11.17	27.19	+2.85	+8.24	+11.11
Mean	-0.22	-0.78	+0.66	1.60	+0.16	+0.46	+0.62

Table 4. *Bird S2. Ca_P and Ca_R lost or gained by bone, the retention of Ca (Ca_A) and egg shell Ca (Ca_S), (g.)*

Day	Laying days				Non-laying days		
	Ca_P	Ca_R	Ca_A	Ca_S	Ca_P	Ca_R	Ca_A
1	—	—	—	—	+0.27	+0.26	+0.53
2	-0.66	-0.55	+0.56	1.69	—	—	—
3	—	—	—	—	-0.44	+1.12	+0.68
4	+0.27	-1.33	+0.77	1.77	—	—	—
5	-0.81	-0.63	+0.50	1.88	—	—	—
6	—	—	—	—	-0.48	+1.29	+0.81
7	+0.27	-1.59	+0.61	1.86	—	—	—
8	-0.66	-0.75	+0.57	1.90	—	—	—
9	—	—	—	—	-0.10	+1.14	+1.04
10	+0.21	-1.13	+0.87	1.72	—	—	—
11	-0.60	-0.58	+0.68	1.78	—	—	—
12	—	—	—	—	+0.35	+0.61	+0.96
13	Omitted because of incomplete data				—	—	—
14	—	—	—	—	+0.25	-0.67	-0.42
15	-0.35	-0.69	+0.79	1.77	—	—	—
16	—	—	—	—	-0.25	+1.25	+1.00
17	-0.19	-0.55	+0.87	1.55	—	—	—
18	+0.23	-1.19	+0.59	1.49	—	—	—
19	—	—	—	—	-0.23	+1.23	+1.00
20	+0.15	-0.89	+0.88	1.56	—	—	—
21	-0.54	-0.19	+0.89	1.55	—	—	—
22	—	—	—	—	-0.10	+0.90	+0.80
23	Omitted because of incomplete data				—	—	—
24	Omitted because of incomplete data				—	—	—
25	—	—	—	—	+0.66	-0.07	+0.59
26	+0.19	-1.82	+0.78	2.31*	—	—	—
27	—	—	—	—	+0.10	+0.78	+0.88
28	+0.35	-0.99	+0.98	1.56	—	—	—
29	-0.64	-0.70	+0.66	1.89*	—	—	—
30	—	—	—	—	+0.64	+0.28	+0.92
31	+0.35	-1.02	+0.99	1.60	—	—	—
32	—	—	—	—	-0.10	+1.08	+0.98
33	+0.25	-0.83	+1.05	1.56	—	—	—
34	-0.52	-0.77	+0.69	1.87*	—	—	—
35	—	—	—	—	+0.83	+0.21	+1.04
Sum	-2.70	-16.20	+13.73	31.34	+1.40	+9.41	+10.81
Mean	-0.15	-0.90	+0.76	1.74	+0.10	+0.67	+0.77

* Indicates double-yolked egg.

Turning now to Tables 2-5 it will be seen that Ca_P shows no definite trend either on days when eggs are laid or on days when no eggs are laid; sometimes the values indicate a loss from the bone and sometimes a gain. The result is that on egg-laying days the daily mean value for Ca_P is, in the case of all four birds, a very small value on the negative side; on the other hand, on days when no eggs are laid the daily mean value for Ca_P shows a small gain in three birds, and in the other, G2, a small loss. The total Ca_P changes for the whole experiment represent a loss in each case.

It would thus appear that the Ca_P in all these four laying hens fluctuates from day to day, that the direction of these fluctuations is not specifically determined by the laying of an egg or by the non-laying of an egg, and that the general trend is towards a loss of Ca_P over the whole period.

A different state of affairs exists however in the case of Ca_R . With only a few exceptions there is quite a large loss of Ca_R on laying days and quite a large gain of Ca_R on non-laying days. Daily mean values for the different birds show gains on non-laying days ranging from 0.46 to 1.20 g. and losses on laying days ranging

Table 5. *Bird G2. Ca_P and Ca_R lost or gained by bone, the retention of Ca (Ca_A) and egg shell Ca (Ca_S), (g.)*

Day	Laying days				Non-laying days		
	Ca_P	Ca_R	Ca_A	Ca_S	Ca_P	Ca_R	Ca_A
1	+0.25	-1.09	+1.18	1.96	—	—	—
2	-0.39	+0.33	+1.77	1.79	—	—	—
3	—	—	—	—	Omitted because of incomplete data		
4	-0.02	-0.73	+1.20	1.90			
5	-0.52	+0.05	+1.40	1.81	—	—	—
6	—	—	—	—	+0.46	+0.73	+1.19
7	—	—	—	—	-0.21	+1.37	+1.16
8	0.00	-0.70	+1.30	1.94	—	—	—
9	-0.48	-0.14	+1.28	1.84	—	—	—
10	—	—	—	—	+0.21	+0.66	+0.87
11	-0.42	+0.19	+1.46	1.64	—	—	—
12	—	—	—	—	+0.37	+0.54	+0.91
13	—	—	—	—	-0.46	+1.68	+1.22
14	+0.19	-1.03	+1.07	1.85	—	—	—
15	—	—	—	—	-0.06	+1.70	+1.64
16	+0.02	-1.05	+0.97	1.93	—	—	—
17	—	—	—	—	-0.42	+1.53	+1.11
18	+0.08	-1.24	+0.77	1.89	—	—	—
19	—	—	—	—	-0.31	+1.42	+1.11
20	+0.17	-0.93	+1.24	1.94	—	—	—
21	—	—	—	—	+0.08	+1.04	+1.12
22	+0.33	-0.45	+0.94	0.99	—	—	—
23	—	—	—	—	-0.54	+1.54	+1.00
24	+0.02	-0.90	+1.09	1.91	—	—	—
25	-0.73	+0.19	+1.50	1.98	—	—	—
26	—	—	—	—	+0.39	+0.38	+0.77
27	—	—	—	—	-0.33	+1.47	+1.14
28	+0.04	-0.92	+0.93	1.76	—	—	—
29	—	—	—	—	-0.52	+1.70	+1.18
30	+0.15	-1.01	+1.10	1.91	—	—	—
31	—	—	—	—	-0.35	+1.45	+1.10
32	0.00	-0.93	+0.90	1.77	—	—	—
33	—	—	—	—	+0.37	+0.37	+0.74
34	—	—	—	—	-0.54	+1.54	+1.00
35	+0.06	-1.13	+0.89	1.90	—	—	—
Sum	-1.25	-11.49	+20.99	32.68	-1.86	+19.12	+17.26
Mean	-0.07	-0.64	+1.17	1.82	-0.12	+1.20	+1.08

from 0.64 to 0.90 g. The total Ca_R changes, however, reveal radical differences in each bird. C2 finished the experiment showing neither a gain nor a loss, S1 and S2 both showed losses and G2 showed a gain.

These findings strongly suggest that it is the Ca_R portion of the bone which undergoes relatively large fluctuations during a laying period and further that the sum total of these fluctuations is considerably affected by the nature of the Ca compound fed to the birds.

The final point to be noted from these Tables is that the daily mean value for the Ca retained (from now on referred to as Ca_A) is almost identical on laying and non-laying days for each individual bird but that there are large variations between the four birds, indicating once more a probable relationship to the kind of Ca compound fed. This naturally leads to a consideration of the relationships between Ca_P , Ca_R and Ca_A .

The daily mean values for each bird for laying days have been collected in Table 6 along with the daily mean values for the Ca of the shell and of the egg contents.

Table 6. *Daily mean values of Ca_P , Ca_R and Ca_A for laying days and the daily mean values for egg shell and egg contents Ca (g.)*

Bird	Ca_P available for egg	Ca_R available for egg	Ca_P + Ca_R	Ca_A available for egg	Ca in shell	Ca in content
C2	0.24	0.84	1.08	0.93	1.96	0.05
S1	0.22	0.78	1.00	0.66	1.60	0.06
S2	0.15	0.90	1.05	0.76	1.74	0.07
G2	0.07	0.64	0.71	1.17	1.82	0.05

Ca_P available for egg is the negative value of Ca_P given in Tables 2-5. This applies also to the values Ca_R and $Ca_P + Ca_R$.

From Table 6 it would appear that on the average, whatever the Ca compound fed, there is a maximum amount of Ca available from the bones ($Ca_P + Ca_R$) which is in the region of 1 g.; this, plus the amount available from the food (Ca_A), decides the amount of Ca in the shell. The small amount of Ca in the egg contents can be neglected here. Thus, provided that the maximum amount of Ca comes from the bones, the final amount of Ca in the shell will be decided by the quantity of Ca retained from the food. This finding lends support to similar ideas expressed by Tyler [1935]. The bird G2 stands alone; in her case only 0.71 g. Ca came from the bones and 1.17 g. from the food. This lowered contribution from the skeleton suggests that in this case the skeleton and the food together could supply more than was required by the shell and therefore a saving was effected on the quantity of Ca withdrawn from the skeleton. The author, using daily averages for a number of birds, has however shown that the maximum amount of Ca which a bird can retain from her food is in the region of 1 g.; therefore the saving effected by food Ca on skeletal reserves is limited.

Briefly then the maximum Ca content of a shell (for Rhode Island Reds) is somewhere about 2 g. and the skeleton will supply about 1 g. and the food about 1 g. If the supply from the food is less than 1 g. then the shell will decrease in Ca content, whilst if the maximum shell value for any individual bird is less than 2 g. and the food supplies 1 g. then there will be an automatic saving of bone Ca.

It must be stressed here however that such statements are generalizations based on average values and when daily values are examined a more intimate relationship between Ca coming from the food (Ca_A) and that coming from the bones ($Ca_P + Ca_R$) is noted.

This is clearly seen when the daily values for ($Ca_P + Ca_R$) are plotted against Ca_A (Fig. 1). There is an obvious reciprocal relationship between the Ca provided by the food and that provided by the skeleton for the purpose of egg shell formation. This relationship holds over the range of values found in this experiment but whether it stretches beyond these ranges is a matter for conjecture. At this stage there is not sufficient evidence to warrant a discussion on the food and bone Ca supplies to the shell, i.e. as to how they interact and what other factors are effective.

The daily mean values of Ca_P , Ca_R and Ca_A for each bird on non-laying days have been collected in Table 7.

In Table 7 the sum of Ca_P and Ca_R equals Ca_A . Once more the maximum retention is about 1 g. Ca but with the bird G2 the effort to keep within the range appears to have been made at the expense of a Ca_P loss. In other words, if the value of Ca_A is below 1 g. then part goes to Ca_P and part to Ca_R , but if the value of Ca_R exceeds 1 g. then there is a loss of Ca_P to keep the value of Ca_A within its limit. This is clearly seen in Fig. 2.

Fig. 1. Relationship between bone calcium ($Ca_P + Ca_R$) and food calcium (Ca_A) for egg shell formation. Four birds C2, S1, S2 and G2.

Table 7. *Daily mean values of Ca_P , Ca_R and Ca_A for non-laying days (g.)*

Bird	Ca_P to bone	Ca_R to bone	Ca_A to bone
C2	0.07	0.84	0.90
S1	0.16	0.46	0.62
S2	0.10	0.67	0.77
G2	-0.12	1.20	1.08

Fig. 2. Relationship of Ca_P to Ca_R gained or lost by the bone. Each point represents the daily mean value for one of the four birds on non-laying days. AB represents the calcium equilibrium line. To the N.E. is a total calcium gain; to the S.W. is a total calcium loss.

Further consideration of Fig. 2 reveals that, whilst in the N.E. quadrant of the graph there is depicted a gain of both Ca_P and Ca_R , in the N.W. quadrant a loss of Ca_P and a gain of Ca_R are represented. Theoretically if the line be continued downwards into the S.E. quadrant there exists the possibility of a gain of Ca_P and a loss of Ca_R , and a slight shifting of the line to the left would lead to a state of affairs in which there would be a loss of both Ca_P and Ca_R . From these very general considerations based on average values there appears to be a reciprocal relationship between Ca_P and Ca_R which relationship can provide theoretically for a loss of P with a gain of Ca, a loss of Ca with a gain of P or a loss or gain of both together. Such a conception is far more elastic and would provide a far more useful mechanism to a bird or animal than the usually accepted idea of a mechanism involving coincident losses or gains of Ca and P. In addition this suggested conception reconciles the two groups of conflicting evidence mentioned earlier in this paper.

It was decided to test the reciprocity of Ca_P and Ca_R more stringently by calculating the correlation coefficients of these values for each of the four birds and for laying and non-laying days. The reason for calculating these values for laying and non-laying days instead of for the whole experiment may be stated briefly. If there is the same reciprocal relationship both on the laying and the non-laying days then this relationship would still be revealed by a high correlation coefficient even when the figures for the whole experiment were taken. On the other hand, if the reciprocal relationship on laying days is different from that on non-laying days then a consideration of the experiment as a whole might give a very low correlation coefficient of no significance. Hence a consideration of correlation coefficients for both laying and non-laying days has no disadvantages but perhaps will reveal something which would otherwise be obscured.

The values of the various correlation coefficients are given in Table 8. All of them are negative and all are highly significant.

Table 8. *Correlation between Ca_P and Ca_R on laying and non-laying days for each bird*

Correlation coefficients (r) and the number of pairs of readings used in their calculation (n).

Bird	Laying		Non-laying	
	r	n	r	n
C2	-0.7883	17	-0.9606	17
S1	-0.6431	17	-0.9149	18
S2	-0.7114	18	-0.7702	14
G2	-0.8403	18	-0.9164	16

Such evidence greatly strengthens the suggested conception. To put this reciprocal relationship into graphical form the regression of Ca_R on Ca_P was calculated and the regression line drawn, and since the correlations are highly significant this line will lie fairly close to the regression line for Ca_P on Ca_R and hence is fairly representative of the true equation relating Ca_P and Ca_R .

These regression lines are shown in Fig. 3 and are drawn within the limits to which they refer, taking as limits the extreme values of Ca_R .

Consideration of Fig. 3 shows that the theoretically possible cases previously outlined are found in practice, namely, coincident gains or losses of Ca and P from the bone or Ca losses with P gains or Ca gains with P losses.

The fact that the regression lines fall into two distinct groups, one for laying and one for non-laying days, suggests that although the reciprocal relationships

exist for Ca_P and Ca_R on both laying and non-laying days yet these relationships are not in the same plane. Since it has been noted already that Ca_P fluctuates but little about zero on both laying and non-laying days the standard of $Ca_P=0$ suggests itself as a basis for comparing the difference in the two reciprocal relationships. On this basis the range of gain of Ca_R on non-laying days is 0.70–1.05 g. and the range of loss of Ca_R on laying days is 0.75–1.05 g.

Fig. 3. Regression lines of Ca_R on Ca_P for the four birds. L=laying days; N=non-laying days. AB represents the calcium equilibrium line. To the N.E. is a total calcium gain; to the S.W. is a total calcium loss.

Therefore the position is this. For non-laying days there is, when $Ca_P=0$, a gain of Ca_R of between 0.70 g. and 1.05 g. depending upon the bird and also upon the supply of Ca. If Ca_P shows a loss then Ca_R shows a larger gain; when Ca_P shows a gain, however, the value of the gain of Ca_R falls away and may even become a loss. On laying days there is, when $Ca_P=0$, a loss of Ca_R of between 0.75 g. and 1.05 g. depending upon the Ca supply and upon the Ca content of the egg shell. If Ca_P shows a gain then Ca_R shows a larger loss, but when Ca_P shows a loss the value of the Ca_R loss falls and may even become a gain.

DISCUSSION

It must first of all be clearly understood that any findings set forth here or any conclusions drawn refer only to the four birds under consideration and to the specific conditions of the experiments.

From the results already discussed there would appear to be a mechanism whereby the laying hen is prevented from losing too much Ca from her skeleton on laying days and from gaining too much on non-laying days. If averages over a whole experiment (35 days) are taken this mechanism seems to work in such a way that the laying hen can prevent the withdrawal of more than about 1.0 g. total Ca from her bones on any one day when an egg is laid and on days when no egg is laid no more than about 1.0 g. total Ca can be laid down in the bones. This

mechanism consists of a reciprocal relationship between Ca_P and Ca_R which acts in such a way that within limits there is a loss or gain of both Ca_P and Ca_R , but as soon as this loss or gain tends to pass beyond these limits in the case of one it is counteracted by an opposite movement of the other.

Much work is required before the mechanism of such changes can be elucidated, but working on the basis that much larger changes occur in the Ca_R than in the Ca_P it might be tentatively suggested that if amounts of Ca_R above a certain maximum are being mobilized then part of that Ca_R will be precipitated as Ca_P leading to a loss of Ca_R and a gain of Ca_P . On the other hand, if amounts of Ca_R above a certain maximum are being laid down, part of this Ca_R must come from a reversion of Ca_P to Ca_R with loss of P, the final result being a gain of Ca_R and a loss of Ca_P .

Whether the Ca_R is made up of CaCO_3 , $\text{Ca}(\text{OH})_2$ or a mixture of both, the large fluctuations of this portion of bone Ca would, in addition to Ca, only liberate or absorb ions which are easily removed or supplied by the body, namely, $(\text{OH})'$ or $(\text{CO}_3)''$. The thesis that $(\text{CO}_3)''$ is the more likely ion for the purpose is supported by that school which believes bone to consist of $\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 and particularly by Klement [1936] who suggests that bone is made up of a hydroxyapatite lattice in which CaCO_3 crystals are deposited.

The final picture one might suggest is therefore a "real" skeleton of $\text{Ca}_3(\text{PO}_4)_2$ which remains fairly constant in amount whilst fluctuating depositions and withdrawals of Ca necessitated during an egg-laying cycle take the form of CaCO_3 whose breakdown or synthesis will not greatly upset the delicate ionic equilibrium. At the same time a limit is set to the total Ca changes by a reciprocal action between the $\text{Ca}_3(\text{PO}_4)_2$ of the "real" skeleton and the easily mobilizable CaCO_3 .

SUMMARY

1. An investigation into the Ca and P metabolism of poultry is described.
2. From the results of these experiments a value for the Ca of the $\text{Ca}_3(\text{PO}_4)_2$ of bone and for the residual Ca of bone gained or lost each day is calculated.
3. It is shown that the residual Ca of the bone is chiefly concerned in egg laying.
4. The relationship of food and bone Ca to egg shell Ca is mentioned.
5. By means of correlation coefficients it is shown that there is a reciprocal relationship between the Ca of calcium phosphate and the residual Ca of bone; the implications of this relationship are discussed.

The author is indebted to the University of Leeds under whose auspices the experimental work was carried out and particularly to Mr J. S. Willcox for his never failing interest. Also to Mr K. L. Robinson for his useful criticism of the ideas embodied in the paper.

REFERENCES

- Bogert & Hastings (1931). *J. biol. Chem.* **94**, 473.
 Brook, Smith & Smith (1934). *J. biol. Chem.* **104**, 141.
 Burns & Henderson (1936). *Biochem. J.* **30**, 1207.
 Common (1938). *J. agric. Sci.* **28**, 347.
 Gassmann (1931). *Hoppe-Seyl. Z.* **201**, 284.
 Klement (1936). *Ber. dtsch. chem. Ges.* **69**, 2232.
 Marek, Wellmann & Urbanyi (1937). *Arch. wiss. prakt. Tierheilk.* **72**, 1.
 Morgulis (1931). *J. biol. Chem.* **93**, 455.
 Richards & Godden (1924). *Analyst*, **49**, 565.
 Tyler (1935). Ph.D. Thesis, Univ. Leeds.