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Calcium Metabolism of Pullets at the Onset of Egg Production, as Influenced by Dietary Calcium Level[1]

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THE skeleton of the laying hen has been shown by many investigators to serve as a calcium store which may be utilized for the formation of the egg shell (Simkiss, 1961). Normally, dietary calcium directly supplies only part of the calcium needed for the formation of the egg shell whereas the rest is withdrawn from the skeleton (Driggers and Comar, 1949). Maintenance of such stores is therefore important for maximum performance of the laying hen.

Ten days prior to onset of egg production, calcium retention of pullets is greatly increased. This increase is commonly associated with the appearance of medullary bone is the marrow cavities of the skeleton (Simkiss, 1961). The possibility that other segments of bone, in addition to medullary bone, may also take part in this pre-laying storage of calcium has not been thoroughly investigated. Neither has it been elucidated whether the origin of calcium for the formation of medullary bone in the maturing pullet is of dietary or intrinsic origin. Govaerts and Dallemagne (1948) found that about half of the phosphorus for medullary bone in the estrogenized pigeon came from the existing bone.

Production of the first few eggs is usually accompanied by a negative calcium balance (Morgan and Mitchell, 1938) regardless of the dietary calcium level (Hurwitz and Griminger, 1960). Taylor and Moore (1954) measured calcium depletion of individual bones at the onset of egg production in pullets fed an essentially calcium-free diet. To the best of our knowledge the depletion of calcium from individual bone segments of pullets at the beginning of egg production has not been studied with normal and high-calcium diets.

In the present study an attempt was made to investigate the storage and depletion of calcium from femur segments at the onset of egg production, of pullets fed diets with either a normal (1.2%) or high (4.1%) calcium level. Calcium-45 was used in order to estimate the degree of bone resorption and renewal, and in order to identify the source of calcium for the formation of medullary bone and its time of formation.

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EXPERIMENTAL RESULTS

General procedure: Calcium and radioactivity measurements were made on plasma from blood obtained by heart puncture. Femurs were removed, *post mortem,* from the birds and cleaned of any adhering tissues. They were then divided into ends and shaft. The shaft was split open and the medullary bone carefully scraped of the cortical bone. The three segments—ends, cortical and medullary—were then analyzed for total dry weight, ash, calcium, phosphorus and radioactivity. Calcium was determined as previously described (Hurwitz and Griminger, 1960), and phosphorus by the method of Gomori (1942). Radioassays were carried out according to standard procedure for calcium-45 (Comar, 1955). For these assays, calcium was precipitated as oxalate out of the HCl solutions. The precipitate was then mounted on a planchett and counted in a thin end window counter. Most samples were prepared at a standard thickness of 8 mg. Ca per sample. When calcium in the samples exceeded this amount, corrections were made from a self-absorption curve. Three 1-minute counts were obtained for each duplicate sample. Actual c.p.m. were 50–250 and 180–800 for trial 1 and trial 2, respectively, with a background count of 10–12 c.p.m. Counting error for each group average (obtained by analysis of variance) was less than 3% in the smallest group, and usually between 1 and 2%. In trial 1 plasma specific activity was low and did not permit accurate counting. Plasma samples of trial 2 were counted with the aid of an automatic sample changer, to a total of 4000 counts per sample.

Analysis of variance of the experimental results was made according to Dixon and Massey (1957).

Trial 1: Seventy-two White Leghorn × RIR pullets, 4 months old, were selected for this study. From 1 day to six weeks of age they were fed a commercial starting ration, and from six weeks to the beginning of the experiment they were fed a commercial growing ration; both rations contained 1.2% calcium.

The pullets were divided into three groups of 24 each: one group to be dosed 7–10 days before the estimated onset of laying, when the existence of medullary bone can be safely assumed, and two groups to be dosed at least 14 days before the estimated date of laying, prior to the formation of medullary bone. The selection of the birds into these groups was done by visual inspection with emphasis on the appearance of the comb. The group to be dosed 7–10 days before laying and one of those to be dosed at least 14 days before laying were given a diet containing 4.1% calcium (high-Ca lot). The remaining group was continued on the growing ration containing 1.2% calcium (low-Ca lot). The composition of the experimental diets is given in Table 1. It is apparent that other than the different calcium levels, there were only slight differences between the diets in their protein, metabolizable energy and phosphorus contents. The low and high calcium rations represent a growing ration (used for pullets until in production), and a high calcium laying ration.

Despite the selection, very few birds actually began to lay within 12 days from dosing date, and thus most birds probably did not contain any medullary bone at the time of dosing. The two groups of the high-Ca lot were therefore combined. However, due to this selection, pullets of the high-Ca lot started to lay on an average of 10 days earlier than those of the low-Ca lot (Table 2).

The birds were kept in individual laying cages placed in an open shed. Feed and water were given *ad libitum.*

TABLE 1.—*Composition of the experimental diets*

Ingredient	Amount, %	
	High-Ca lot	Low-C lot
Soybean meal (45% protein)	19.00	13.00
Fish meal (60% protein)	3.00	3.00
Alfalfa meal, dehydrated	5.00	3.50
Wheat bran	5.00	10.00
Ground milo	21.95	66.35
Ground yellow corn	35.00	—
Soapstock soya	1.00	1.00
Vitamin mixture[1]	.25	.25
Mineral mixture[2]	.30	.30
Dicalcium phosphate	1.50	1.40
Limestone	8.00	1.20
Composition		
Crude protein, calculated, %	17.1	16.4
Metabolizable energy, calculated, Cal./100g.	262	276
Calcium, assayed, %	4.1	1.2
Phosphorus, assayed, %	.67	.68

[1] Supplying per kg. of ration: High-Ca lot—vitamin A, 10,000 I.U.; vitamin D_3, 1,400 I.C.U.; riboflavin, 3 mg.; pantothenic acid, 3 mg.; niacin, 10 mg.; choline chloride, 400 mg.; B_{12}, 8 mcg.; BHT, 125 mg.; Low-Ca lot—vitamin A, 7,500 I.U.; vitamin D_3, 1,000 I.C.U.; vitamin E, 1 I.U.; menadione, 2 mg.; riboflavin, 4 mg.; pantothenic acid, 8 mg.; niacin, 20 mg.; choline chloride, 150 mg.; B_{12}, 10 mcg.; BHT, 125 g.

[2] Mostly sodium chloride. Also supplying in mg. per kg. of ration: Mn, 60; Zn, 50; I, 1.2; Co, 0.2; Cu, 2; Fe, 25.

Three days after starting the feeding of the experimental diets, each pullet was given intravenously 2 μc. of $Ca^{45}Cl_2$ of specific activity of 1 μc./mg. Ca.

Birds were killed just prior to the onset of egg production (0 eggs), and after laying 1, 2 and 5 eggs (laying groups). The selection of the 0-egg group was made on the basis of external signs and after killing it was ascertained, by the size of the follicles, that the birds had been about to lay within 1–3 days. The killing schedule was arranged so that birds from each laying group were killed at different times during the entire experimental period, in order to rule out any effect of time within any calcium lot. This resulted in a similar average time interval from dosing to killing (Table 2). During the course of the experiment, 5 birds had died of leucosis and an additional five had not started to lay 40 days after dosing and were therefore eliminated from the experiment. The number of birds in each experimental group is given in Table 2.

There was no significant difference in body weight between the groups. Average

TABLE 2.—*The effect of dietary calcium level and number of eggs laid at the onset of egg production on plasma calcium content*

	Dietary Ca level, %	Eggs laid before death				Avg.[1]
		0	1	2	5	
Number of birds	4.1	13	10	9	9	
	1.2	7	5	6	3	
Total[2]		20	15	15	12	
Time of dosing to killing, days[3]	4.1	17	19	14	23	18
	1.2	31	29	25	25	28
Avg.		22	22	18	23	21
Plasma calcium, mg./100 ml.[4]	4.1	32.8	27.7	28.6	26.4	28.9
	1.2	25.7	24.8	21.8	21.1	23.6
Avg.		30.3	26.7	26.2	24.9	27.1

[1] All averages are weighted according to the number of birds.

[2] The difference between the two lots is due mainly to the selection of more sexually mature pullets for the high-Ca lot. For additional explanation, see text.

[3] Ten out of the 72 experimental birds are not included due to death and lack of production.

[4] Standard error obtained from the error term in the analysis of variance was variable according to the number of birds per group. It ranged from 1.7 to 3.5 for $n=3$ and $n=13$, respectively.

TABLE 3.—*Calcium content of femur segments from pullets receiving two levels of dietary calcium 3–4 weeks before the onset of egg production*

Femur segment	Dietary calcium level, %	Eggs laid before death				Avg.[1]
		0	1	2	5	
				mg.		
Ends	4.1	622	590	563	541	584
	1.2	567	530	485	342	502
Avg.[2]		603	570	532	491	539
Shaft, cortical	4.1	506	510	449	490	491
	1.2	456	459	439	406	445
Avg.		489	493	445	469	461
Shaft, medullary	4.1	109	156	132	170	139
	1.2	124	184	131	87	135
Avg.		114	165	132	149	138
Total femur	4.1	1,237	1,256	1,145	1,201	1,214
	1.2	1,148	1,173	1,056	836	1,083
Avg.		1,206	1,229	1,109	1,109	1,169

[1] All averages are weighted, according to the number of birds shown in Table 2.
[2] Standard error is 29–62, 18–38, and 14–30 for ends, cortical and medullary segments, respectively. For explanation see footnote 4, Table 2.

body weight ranged between 1791 and 1908 g., and was 1862 and 1845 g., for the high and low-Ca lots, respectively.

Plasma calcium concentration is shown in Table 2. The plasma of pullets of the high-Ca lot had a higher calcium content than that of the low-Ca lot ($P < .05$). Egg laying tended to reduce plasma calcium, although nonsignificantly.

Ash percentage in the three femur segments was influenced neither by dietary calcium nor by egg laying. It averaged 47.8%, 66.0% and 33.6% in ends, cortical and medullary segments, respectively. Percentage ash of the total femur averaged 51.5% and 50.3% for the high and low-Ca lots, respectively.

Average calcium content of the various bone segments is given in Table 3. Femurs of the high-Ca lot contained more calcium than those of the low-Ca lot ($P < .01$). This difference was found in the ends and cortical segment, but not in the medullary segment, and was already apparent before the onset of egg production (0-egg group). Egg laying reduced the calcium content of the entire bone in general, and of the ends in particular ($P < .05$). This reduction was especially apparent in the low-Ca lot. The calcium content of the cortical and the medullary segments showed no change due to egg laying, although a reduction tendency could be noticed in the calcium of the cortical segment in the low-Ca lot.

Ca/P ratio of the femur segments is shown in Table 4. Bones from the high-Ca lot had a higher Ca/P ratio than those of the low-Ca lot, the difference being small but highly significant ($P < .01$). In the cortical segment only, this difference was not significant. Egg laying did not influence the Ca/P ratio, significantly, in any of the three bone segments.

The percentage of the injected calcium-45 dose retained in the various bone segments is given in Table 5. The high level of dietary calcium reduced the retention of the radioisotope in the ends and cortical segment ($P < .01$) but not in the medullary segment ($P > .05$). Egg laying re-

TABLE 4.—*Ca/P weight ratio of femur segments from pullets receiving two levels of dietary calcium beginning with 3-4 weeks before onset of egg production*

Femur segment	Dietary calcium level, %	Eggs laid before death				Avg.[1]
		0	1	2	5	
		mg. Ca/mg. P				
Ends	4.1	2.22	2.24	2.26	2.24	2.24
	1.2	2.19	2.20	2.14	2.10	2.17
Avg.[2]		2.21	2.23	2.21	2.20	2.21
Shaft, cortical	4.1	2.19	2.19	2.23	2.22	2.21
	1.2	2.18	2.21	2.16	2.20	2.18
Avg.		2.19	2.20	2.20	2.21	2.20
Shaft, medullary	4.1	2.18	2.17	2.20	2.21	2.19
	1.2	2.12	2.19	2.11	2.06	2.12
Avg.		2.16	2.18	2.16	2.18	2.17
Total femur	4.1	2.20	2.20	2.23	2.22	2.21
	1.2	2.16	2.20	2.14	2.21	2.16
Avg.		2.18	2.20	2.19	2.20	2.19

[1] All averages are weighted, according to the number of birds shown in Table 2.
[2] Standard error was 0.015–0.031, 0.017–0.035 and 0.026–0.055, for ends, cortical and medullary segments, respectively. For explanation, see footnote 4, Table 2.

duced percentage dose of calcium-45 in the ends and cortical segment ($P < .05$). There was no significant change in percentage dose with egg laying, in the medullary segment, although the 5-egg group of the low-Ca lot was reduced.

Specific activity is shown in Table 6. As was the case with percentage dose, specific activity was higher in the ends and the cortical segments of the low-Ca lot ($P < .01$). Egg laying somewhat reduced specific activity in the femur segments, but not

TABLE 5.—*Percent of the dose of calcium-45 retained in femur segments of pullets fed two levels of dietary calcium beginning with 3–4 weeks before onset of egg production*[1]

Femur segment	Dietary calcium level, %	Eggs laid before death				Avg.[2]
		0	1	2	5	
		% dose				
Ends	4.1	2.06	1.93	1.68	1.48	1.82
	1.2	2.77	2.41	2.29	1.67	2.39
Avg.[3]		2.31	2.09	1.92	1.53	2.01
Shaft, cortical	4.1	.94	1.19	.73	.85	.94
	1.2	1.67	1.52	1.18	1.16	1.42
Avg.		1.20	1.30	.91	.93	1.10
Shaft, medullary	4.1	.35	.47	.43	.36	.40
	1.2	.45	.65	.47	.23	.47
Avg.		.39	.53	.44	.33	.42
Total femur	4.1	3.36	3.59	2.83	2.70	3.16
	1.2	4.89	4.58	3.94	3.06	4.28
Avg.		3.90	3.92	3.28	2.79	3.54

[1] A single tracer dose was injected intravenously 3 days after starting the experimental diet feeding.
[2] All averages are weighted, according to the number of birds shown in Table 2.
[3] Standard error was 0.14–0.30, 0.11–0.23 and 0.06–0.13 for ends, cortical and medullary segments, respectively. For explanation, see footnote 4, Table 2.

TABLE 6.—*Specific activity of femur segments from pullets fed two levels of dietary calcium beginning with 3–4 weeks before the onset of egg production*[1]

Femur segment	Dietary calcium level, %	Eggs laid before death				Avg.[2]
		0	1	2	5	
				% dose/g. Ca		
Ends	4.1	3.48	3.31	3.06	2.82	3.20
	1.2	5.05	4.68	4.79	4.96	4.87
Avg.[3]		4.03	3.77	3.75	3.35	3.77
Shaft, cortical	4.1	1.89	2.29	1.69	1.76	1.91
	1.2	3.73	3.26	2.70	2.88	3.20
Avg.		2.53	2.61	2.09	2.04	2.35
Shaft, medullary	4.1	3.21	3.23	3.36	1.77	2.93
	1.2	3.85	4.24	3.73	2.73	3.75
Avg.		3.43	3.56	3.51	2.01	3.21
Total femur	4.1	3.12	2.85	2.53	2.30	2.75
	1.2	3.96	3.97	3.75	4.43	3.97
Avg.		3.41	3.23	3.02	2.83	3.16

[1] See footnote 1, Table 5.
[2] All averages are weighted, according to the number of birds shown in Table 2.
[3] Standard error was 0.30–0.61, 0.24–0.49, and 0.46–0.95 for ends, cortical and medullary respectively. For explanation, see footnote 4, Table 2.

significantly. The tendency was apparent in the ends of the high-Ca lot but not in the low-Ca lot. In the cortical segment there was no uniform pattern, and in the medullary segment, only the 5-egg group exhibited a lower specific activity.

Trail 2. The purpose of this trial was: (a) to determine the time effect on the retention of calcium-45 in the maturing pullets; (b) to estimate the time interval between the beginning of medullary bone formation and egg laying, and (c) to clarify the relationship between the specific activity of blood plasma and medullary bone, and to identify the immediate source of calcium for medullary bone formation.

Twenty White Leghorn pullets, 4½ months old, were used for this trial. They were raised as described above, and given the high-Ca diet (Table 1), two weeks before the start of the experiment. The pullets were divided into three groups: four birds were to be dosed within one week before egg laying; representatives of this group killed at the time of dosing showed a considerable amount of medullary bone in their femurs. Four other birds were to be dosed between one and two weeks before egg laying. The remaining 12 birds were to be dosed more than two weeks before egg laying; representatives of the last group had no medullary bone in their femurs when killed at the time of dosing. The birds were given approximately 7 μc. of calcium-45, with a specific activity of 2 μc./mg. Ca. During the entire experiment they were kept as described for trial 1. The birds were bled and killed after laying their first (and only) egg, and calcium and radioactivity estimated in their blood plasma, egg shell and femur segments. Specific activity, calculated from these measurements, is shown in Figure 1.

Specific activity of the egg shell was rapidly reduced from 2 to 13 days after dosing. From 16 days after dosing, specific activity of the egg shell seems to have reached a relatively constant value. Specific activity of the plasma (taken after the bird had laid) was at the start much lower than that of the shell, the difference being minimized 13 days after dosing.

Specific activity of the ends and cortical segments of the femur showed slightly increasing values from 2 to 12 days but remained relatively unchanged to the end of the experiment. On the other hand, specific activity of the medullary segment declined markedly, about 14 days after dosing, from levels higher to levels lower than those of the ends. Similar to other components analyzed, medullary bone specific activity remained constant from 14 days to the end of the experiment.

Except for samples obtained 2 and 5 days after dosing, specific activity of the ends and medullary segment was usually higher than that of egg shell and plasma. Specific activity of the cortical segment being lower than that of the other segments,

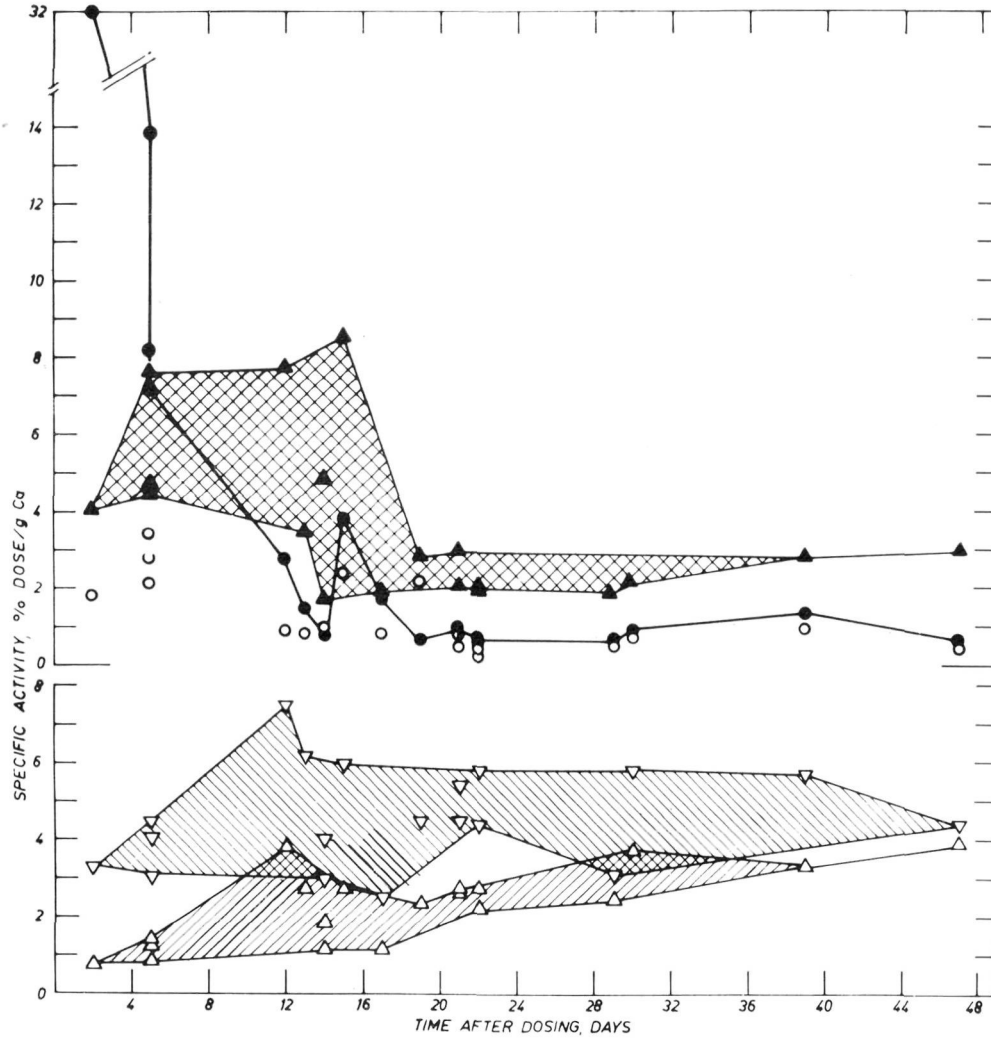

FIG. 1. Upper diagram: specific activity of the first egg shell (●), blood plasma (○) and femur medullary segment (▲); lower diagram: specific activity of the femur ends (▽) and cortical segment (△). Results of pullets dosed with calcium-45 before the onset of egg production. The shaded areas are those included within the range of specific activity of the respective bone segment.

exceeded the specific activity of shells and plasma after about 2 weeks.

DISCUSSION

The influence of the time interval from losing to laying on the retention of calcium-45 in plasma calcium and bone segments, and its deposition in the egg shell, is apparent from results of trial 2. Shells of eggs laid 2 and 5 days after dosing had specific activity higher than that of plasma. Since shell specific activity should be the same as that of the plasma during the period of deposition, plasma specific activity was probably higher before shell formation and the production of the egg shell reduced the specific activity of the plasma. However, 12 days after dosing there occurred some equilibrium, and plasma specific activity was similar to the egg shell. With regard to the relationship between the egg shell and blood, it should be remembered that the plasma sample was obtained after the egg shell had been deposited.

Specific activity of femur ends and cortical segments did not change appreciably after 12 days, following a slightly increase from 2 to 12 days. In contrast to these bone segments, there was a rapid fall in specific activity of the medullary segment between the 14th and the 16th day after dosing. It seems, therefore, that medullary bone was present or was in a stage of rapid formation at dosing time in the pullets killed up to 14 days after dosing. It should be remembered that representatives of the group that started laying 17 days after dosing did not have any medullary bone in their femurs. These findings suggest 14–16 days as the time interval between the beginning of medullary bone formation and the onset of egg production. These figures agree well with the 2-week period in which increased daily calcium retention was observed (Common, 1932).

The comparison of the results of the two calcium lots in trial 1 seems to be valid despite the 10-day difference in the time interval from dosing to killing, since in trial 2 the ends and cortical segment did not change significantly from 12 days on, and probably few of the high-Ca lot had any medullary bone in their femurs at the time of injection.

Plasma calcium (Table 2) was found to be influenced by dietary calcium, in agreement with the observations of Mueller (1958). Egg laying tended to reduce plasma calcium, possibly due to transfer of this element to the egg shell.

The Ca/P ratio of ends and medullary segments of the femur was increased by feeding the high-calcium diet (Table 5). Similarly, Taylor and Moore (1958) reduced this ratio in pullets by feeding high phosphorus diets. It seems, therefore, that the Ca/P ratio in the bones of maturing pullets may be manipulated by dietary minerals in similarity to chicks (Hurwitz and Griminger, 1961).

High dietary calcium promoted a greater pre-laying storage of calcium in the femur, and reduced the calcium depletion associated with early egg laying (Table 3). This finding is in agreement with previous results obtained by balance techniques (Hurwitz and Griminger, 1960).

Among the three femur segments studied, the ends proved to be a primary site of storage of available calcium (Table 3); differences were found between the calcium lots in pre-laying storage of calcium in this segment, which lost calcium during successive egg laying. Although the cortical segment of the high-Ca birds stored more calcium than that of the low-Ca birds, this segment did not lose calcium during early egg laying and therefore can not be considered as an immediate available calcium reserve. In more severe calcium depletion, however, cortical bone may also be called upon to supply calcium to the egg shell.

Storage and depletion of calcium from bone was attained through the addition of new bone and whole bone resorption, respectively. There were no differences in the degree of mineralization (percentage ash) among the various groups, but differences were found in bone dry weight. These results are similar to those of Taylor and Moore (1954), and to previous results (Hurwitz and Griminger, 1961) obtained from laying hens after severe calcium depletion.

Similarly to findings in rats (Wasserman *et al.*, 1957), dietary calcium reduced the retention of radiocalcium in bones of pullets. Calcium was probably metabolized more efficiently in the low-Ca lot, which thus retained more of the radiocalcium.

The decrease in calcium-45 in bones due to egg laying, may result from exchange (Hurwitz, 1964a), resorption of a bone fraction with a high specific activity, or bone replacement from a low specific activity source of calcium (diet via plasma). Under our experimental conditions, an exchange reaction seems improbable because of the relatively long period from dosing to killing. In trial 2 it was found that after a period of 12 days, plasma specific activity was quite constant and lower than that of bones. This would indicate that little labelling remained in the exchangeable compartment, and that the reduction of radioactivity in bones resulted primarily from bone resorption.

In the ends, there was a highly significant reduction in percentage dose of calcium-45, but a rather slight reduction in specific activity (Tables 5 and 6), which could be observed only in the high-Ca lot. This finding, together with the above-mentioned reduction in total calcium of this segment, indicates a decided bone resorption with little replacement. Some bone replacement was, however, indicated by the reduced specific activity in the high-Ca lot.

There was probably some resorption in the cortical segment due to egg laying, as indicated by the significant decrease in percentage dose. The latter seems to be component of a small reduction in calcium content and percentage dose, which did not reach statistical significance.

Changes in medullary bone deserve a special comment. Both trials indicate a high specific activity in medullary bone, long after the isotope administration. In trial 2, medullary bone specific activity was 2–3 times higher than that of the plasma even after close to 7 weeks after dosing. This finding may lead to the conclusion that medullary bone in the pullet may derive at least part of its calcium from adjacent structural bone, which would have a higher specific activity than the blood plasma. In this connection, Govaerts and Dallemagne (1948) also concluded that about 50% of the phosphorus for the formation of medullary bone in the estrogenized male pigeon was derived from structural bone.

Many investigators (Simkiss, 1961) found medullary bone to be highly labile, undergoing intensive resorption during periods of egg shell formation. Furthermore, bones from hens constantly fed with a calcium-45 labelled diet indicate a rapid turnover rate of medullary-bone calcium greatly exceeding other bone segments (Hurwitz, 1964b). It therefore seems unlikely that in the present trial medullary bone had not undergone any changes with egg laying, although no marked change had apparently taken place in either its calcium content or its specific activity (Tables 5 and 6). It seems, rather, that calcium lost from the medullary bone of the young pullets during shell formation, was replenished by a high-specific activity calcium from structural bone, rendering the activity relatively unchanged. This hypothesis would also ex-

plain why formation of medullary bone was found unaffected by dietary calcium, whereas calcium in the other bone segments increased with dietary calcium supplementation (Table 3). It is also in agreement with the results of Taylor and Moore (1954) who found medullary bone to be maintained under conditions of calcium depletion despite a marked fall in cortical ash. This was considered by Taylor and Moore as a support of the common view that medullary bone is a source of readily available calcium for the egg shell. This source is utilized constantly, whereas reserves of calcium (*i.e.* ends) are called upon during conditions of calcium insufficiency.

SUMMARY

Calcium storage and depletion, and retention of Ca^{45} in the different segments of the femur and in the blood plasma, were studied in pullets at the onset of egg production. The birds were fed diets containing either 4.1% or 1.2% calcium, and received a single dose of Ca^{45} 3 days and two weeks for trial 1 and 2, respectively, after being started on the experimental rations. The high-Ca diet promoted a higher plasma calcium concentration and a greater pre-laying storage of calcium in the ends and cortical segments, but not in medullary segment. It increased Ca/P ratio in all segments, but reduced the retention of Ca^{45} in ends and cortical segment. Dietary calcium did not influence percentage ash. Production of the first 5 eggs resulted in a loss of stable calcium and Ca^{45} from the ends which was more severe in the low-Ca birds; no such depletion occurred in the medullary segment, but the cortical segment lost some Ca^{45}. Specific activity of the ends changed only slightly, indicating little replacement of the calcium depleted. The results suggest that medullary bone in the pullet is formed about 2 weeks before egg laying and its calcium largely derived from structural bone. The importance of each segment as a calcium storage site is discussed.

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Effect of Sex Upon the Distribution of Zinc in the Adult Fowl[1]

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KNOWLEDGE of the factors which affect the distribution of zinc in tissues is necessary to elucidate the metabolism of this mineral. Gunn and Gould (1956) reported that the zinc content of the prostate of the rat was influenced by the sex hormones. Testosterone propionate and the gonadotrophins were found by Millar *et al.* (1957) to produce marked increases in zinc concentration and zinc-65 uptake of prostate glands of rats. Estrogen did not change the zinc content of these glands.

No information was available for the effect of the sex hormones upon the zinc concentration of the general body tissues or the reproductive tissues of the fowl, therefore, this study was undertaken to determine these effects.

PROCEDURE

In the first experiment, five female and one male SCWL birds were assigned to each of five lots. Each lot was fed one of the following semipurified diets: casein basal, casein basal supplemented with 60 p.p.m. zinc, isolated soybean protein basal, isolated soybean protein basal supplemented with 60 p.p.m. zinc, or a sesame meal ration. The formulas used for the first four rations were those reported by Keinholz *et al.* (1961). The sesame meal ration was modified from the one reported by Lease *et al.* (1960) by decreasing the protein level to 16% and adding calcium to 2.25% of the ration. The birds were six months old at the start of the experiment. At the end of the nine-month experimental period during which individual egg production records were kept on the females the birds were sacrificed, bled, the tissues removed and quick frozen for later zinc analyses.

In the second experiment, femurs from two lots of five male and five female adult sex-linked birds each were used. The birds from one lot had been reared from one day to 16 months of age on diets containing sesame meal, while the birds from the other lot had been reared under similar conditions with a diet containing sesame meal supplemented with 120 p.p.m. of zinc.

In the third experiment, two lots of adult birds, similar to those used in experiment 2, were given 50 μc. of $Zn^{65}Cl_2$. Two of the males and two of the females from each lot were given the tracer as an intravenous injection. An oral dose of the tracer was

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