PART ONE. PHOSPHATASE AND INORGANIC PHOSPHORUS IN THE PLASMA AND WHOLE BLOOD OF THE FOWL

PART TWO. FLUCTUATIONS OF THE PHOSPHATASE AND INORGANIC PHOSPHORUS IN THE BLOOD OF THE LAYING HEN DURING THE PERIOD OF EGG FORMATION

by

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B. S., Kansas State College of
Agriculture and Applied Science, 1935

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1938

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PART ONE. PHOSPHATASE AND INORGANIC PHOSPHORUS IN THE PLASMA AND WHOLE BLOOD OF THE FOWL

INTRODUCTION AND REVIEW OF LITERATURE

Recognition of the fact that the estimation of the serum (or plasma) phosphatase gives a somewhat earlier indication of the severity of rickets and the course of healing than is shown by the concentrations of calcium and phosphorus in the serum has resulted in considerable work on this enzyme.

Though relatively few reports are available which deal with the blood phosphatase of the fowl, our knowledge of the enzyme has been considerably broadened by numerous studies on mammals. The possible function of phosphatase in the growth and disease of bone has been reviewed by Kay (1932) and has received attention in a symposium on ossification[1]. More recently our knowledge of the significance of the enzyme has been reviewed by Cristol and Cayla (1936), and Morris and Peden (1937). As pointed out in

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[1] Rept., 1933, Liecester Meeting British Association Advancement Science, Secretary's Transcription, p. 531.

these reviews, clinical use is now being made of such studies. It has been shown by Hall and King (1931), and King and Hall (1931) that tissue phosphatase extracts of the fowl react in a manner similar to those of mammals.

Recently Common (1934, 1936) has studied the serum phosphatase activity in (a) normal birds from hatching to maturity, (b) laying birds receiving vitamin D supplement in the ration, (c) laying birds receiving high and low calcium rations, and (d) rachitic chicks. He also studied a group of normal birds of various stages of development. Since so little is known concerning phosphatase in the fowl, it might be well to summarize his findings.

In his preliminary work, he found that (a) the values for laying hens were higher than those for cocks, (b) the values for pullets that had never laid were of the same order as for cocks, (c) there was no direct connection observed between serum phosphatase and intensity of egg production, (d) adult layers and non-layers gave values not very different, (e) individual birds had serum phosphatase values of the same order three months after the first determination, and (f) there was considerable variation from bird to bird of the same group. In later exper-

iments, he found that there was no significant difference in the values between the sexes from the twenty-sixth day till the onset of laying. Then as the birds matured the values for cocks decreased and were of approximately the same order as found in an earlier experiment. The average for pullets was much higher; however, this value was arrived at by averaging widely varying results, some of which were as low as found in the preliminary work. While Radiostol exercised no appreciable effect on the serum phosphatase of normal birds, severe rickets caused greatly raised phosphatase values. Birds on a low calcium diet showed rapid increase of serum phosphatase on starting to lay.

In their studies of the phosphatase of the adult fowl, Auchinachie and Emslie (1934) found that young animals have higher values than adults. They likewise found increased phosphatase values in cases of rickets and other bone disorders, and there seemed to be no relation between laying records and phosphatase values.

The same workers (op. cit.) have raised the question as to whether estimations of plasma (or serum) phosphatase activity will give any indication of faulty calcium and phosphorus metabolism in fowls, or whether there is nor-

mally such variation that phosphatase estimations have limited significance. When pullets were fed different amounts of vitamin D there was no significant change in inorganic phosphorus; the serum values also showed the normal range for mature hens. The plasma phosphatase, however, showed a wide and unexplainable variation. It was concluded by them, as well as by Common (1936), that there was no relation between the vitamin content of the diet and the activity of the plasma phosphatase. These workers feel, nevertheless, that there is more than a mere suggestion that an abnormally high plasma phosphatase activity does indicate that conditions are not optimum for calcium and phosphorus metabolism.

In view of the wide normal range of plasma phosphatase reported by Common (1934, 1936), and Auchinachie and Emslie (1934), it would appear that further studies designed to determine the factors responsible for these variations would be a valuable and necessary preliminary to subsequent vitamin and mineral studies. In this study, data on phosphorus and phosphatase levels of the plasma and whole blood of the male and female birds at five and sixteen months of age will be considered.

PROCEDURE

All of the birds were bled from the wing vein. After removing the feathers covering the vein, a small v-shaped cut was made in the skin directly over it. A small slightly curved needle was inserted into the vein with the point towards the distal end of the wing. The blood was drawn at such a rate that the vein would not collapse. The syringe that was used was washed each time with a small amount of two per cent oxalate solution, and the blood was immediately transferred to tubes containing a small amount of oxalate which had been crystallized in the tube. The tubes were tilted several times to mix the blood and the anti-coagulant; then they were placed in ice water until analysis could be made. The amount of blood necessary depended upon the particular type of analysis that was to be made - usually three to six cubic centimeters were drawn.

The analyses were made as soon as possible after collecting the samples. Phosphatase was determined by the method of Bodansky (1931, 1933) with a slightly different procedure in that in the determination of the inorganic phosphate (an integral part of the phosphatase determina-

tion) a modification of the Fiske and Subbarow method was used. (Koch 1934, p. 150). In the preparation of the plasma, a well mixed sample of the oxalated blood was transferred to a small tube and centrifuged for ten minutes.

Early in this work, it was discovered that frequently there was a significant difference between the phosphorus and phosphatase values obtained on plasma and oxalated blood at the same bleeding. A detailed study of these differences and their variations with the age and sex of the bird gave interesting results. Plasma and whole blood values shown in Series 1, Table 1 were obtained from single bleedings. The results shown in Series 2, Table 1 are similar except that in some cases plasma and whole blood samples were obtained from bleedings of different birds; these have been averaged with the values from Series 1 where the birds were from the same flock and thus comparable. The birds used were strictly normal birds on normal feed with access to green range and sunshine, except in one case as shall be noted.

This work was carried out in the months of June, July, and August.

Table 1. The range of phosphorus and phosphatase of whole blood and plasma.

Series	Variety	Sex	Age in months	No. in group	Phosphorus mg/100 cc.				Phosphatase (Bodansky)			
					Whole blood		Plasma		Whole blood		Plasma	
					Range	Average	Range	Average	Range	Average	Range	Average
I	R.I.R.	Male	5	8	3.9-5.1	4.6	4.2-6.0	5.3	6.2-21.8	15.3	4.5-35.6	21.9
	R.I.R.	Male	16	2	2.9-3.4	3.2	2.7-4.5	3.6	7.9- 9.1	8.5	6.9- 8.6	7.8
	R.I.R.	Female	5	6	3.3-5.1	4.3	3.5-5.2	4.5	9.0-31.5	19.5	10.4-41.7	26.5
	R.I.R.	Female	16	7	3.1-3.9	3.6	2.9-4.2	3.9	7.2-25.1	12.2	5.5-32.4	14.4
	W.L.	Female	5	3	4.2-5.0	4.7	4.7-5.7	5.3	11.7-14.2	12.7	10.4-22.0	15.9
	W.L.*	Female	16	7	2.9-6.1	4.0	2.9-7.3	4.8	6.9-11.4	8.0	5.3-13.1	7.7
	W.W.	Male	5	10	1.8-4.9	3.4	1.7-3.9	2.9	7.9-21.0	13.2	5.9-28.3	15.9
II	R.I.R.	Male	5	23	3.9-6.5	5.0			6.2-24.4	17.6		
	R.I.R.	Male	5	10			4.2-6.0	5.2			4.5-35.6	19.7
	R.I.R.	Female	16	15	3.1-5.8	4.1			7.2-25.1	12.4		
	R.I.R.	Female	16	9			2.9-5.0	3.8			5.5-32.4	14.0
	W.L.	Male	16	4	1.8-3.6	2.7			7.2-10.8	8.8		
	W.L.	Male	16	2			1.7-2.5	2.1			11.5-17.6	14.6
	W.L.*	Female	16	13	2.9-6.1	4.2			6.1-11.4	7.2		
	W.L.**	Female	16	40			2.9-7.5	4.4			2.2-20.6	6.8
	M.C.B.	Male	16	7	2.3-5.3	3.5			7.3-16.3	10.1		
	M.C.B.	Male	16	3			2.7-3.8	3.3			4.7- 6.7	5.8
	Average values for 5 and 16 month old males and females											
		Male	5	33/20***	1.8-6.5	4.5	1.7-6.0	4.0	6.2-24.4	16.2	4.5-35.6	17.8
		Female	5	9/9	3.3-5.1	4.4	3.5-5.7	4.7	9.0-31.5	17.2	10.4-41.7	22.9
		Male	16	13/7	1.8-5.3	3.2	1.7-4.5	3.0	7.2-16.3	9.4	4.7-17.6	8.9
		Female	16	28/49	3.1-6.1	4.2	2.9-7.5	4.3	6.1-25.1	9.9	2.2-32.4	8.1

*Normal laying hens.
**Part layers and part out of production at this time.
***First figure refers to number in the whole blood group; the second to the number in the plasma group.
R.I.R. - Rhode Island Red. W.L. - White Leghorn. W.W. - White Wyandotte. M.C.B. - Mixed Cross Breeds.

DISCUSSION OF RESULTS

The average plasma phosphatase value for five-month-old cockerels was 10 per cent higher than the average for whole blood. For pullets of the same age, the plasma phosphatase was 33 per cent higher. Similar comparisons for birds of sixteen months of age, however, showed a different trend. In fact, plasma phosphatase values were lower than those for whole blood of both males and females.

This tendency in the younger birds for the plasma to have a higher phosphatase value than whole blood is interesting in view of the work of Auchinachie and Emslie (1934) who found no significant difference when comparisons were made of the phosphatase activity of oxalated blood plasma, "native plasma", and serum. These workers, however, did not include whole blood in their studies.

Very striking differences were also found (Table 1) between the phosphatase values for birds of different ages, irrespective of whether the determinations were carried out on plasma or on whole blood. Average phosphatase values were from 58 to 150 per cent (average 116 per cent) higher for birds at five months than for birds of corresponding

breed and sex at sixteen months. These results are in agreement with those of Auchinachie and Emslie (1934) who found that Rhode Island Red cocks at seven months had an average plasma phosphatase value of 9.6 units[2], but thirteen-month-old birds had a value 50 per cent lower, or 4.8 units. These workers did not find a wide individual variation in the values obtained for cockerels, especially after they had reached maturity, while pullets showed a somewhat wider range. In the present study, however, both cockerels and pullets showed a wide individual variation; while normal laying hens had only a slightly lower but much more variable phosphatase value than cocks of the same age.

In regard to the variations of phosphatase with age, Common (1936) reports that for day-old chicks a high initial value 80-90 units increased to a maximum of about 110 units at 10-12 days, followed by a sharp decline to 40-50 units at three weeks, after which the values for male birds fell regularly until maturity. At five months of age, he reports values for White Wyandotte males and females of 23.9 and 23.6 respectively. The values are somewhat of the same order as those found in the present study on Rhode

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[2] Kay units have been converted to Bodansky units (Auchinachie and Emslie, 1934).

Island Reds.

A comparison of phosphatase values found in various studies is interesting. Auchinachie and Emslie (1934) report a value of 4.2 for White Leghorns at fifteen months, 6.6 for Rhode Island Reds at fifteen months, and 9.3 for White Wyandottes at seventeen months. For 32 normal White Wyandotte laying hens, Common reports a value of 17.1. In the present work, a value of 6.8 was found for White Leghorns, and 14.0 for Rhode Island Reds at sixteen months of age. In view of these facts, there is an indication that the values for the heavier breed hens (i.e., White Wyandotte and Rhode Island Red) are somewhat higher than those for the White Leghorn hens.

Average plasma inorganic phosphorus values, unlike phosphatase, were not significantly different from the values obtained on whole blood. However, the plasma phosphorus value for sixteen-month-old males was 25 per cent lower, and for females only 9 per cent lower than at five months of age.

Roche and Filippi (1937) found that in young rats, the phosphatase system of the leg bones is fully activated and the addition of Mg ions has no effect; whereas in the adult

the enzyme is present but only partly activated, so that in the latter case the addition of Mg ions produced a large increase of activity. They conclude that the enzyme is present in the latter case but the catalyst is necessary to obtain full activity. They further show that the variations in response of the tissue phosphatases are mainly due to the presence of inhibitors and impurities. It may be that the lowered enzyme of the older birds is due to the lack of an activator or the presence of an inhibitor. On the other hand, the physiological system may be such that the enzyme is not maintained in the body in large amounts as maturity is reached. It has been pointed out by Kay (1932) that the phosphatase may be involved in both the calcification and the demineralization of bone. Mature birds, whose skeletal tissues have passed the period of active growth, may not have the need for phosphatase as required by growing birds.

In the course of this work, advantage was taken of an opportunity to study the whole blood phosphatase of a group of ten-week-old White Leghorn chicks which had been kept indoors since hatching on a ration deficient in vitamin A but adequate in other respects. The values proved to be practically identical with those obtained on controls

receiving an adequate ration (14.9 and 15.0). When the birds which had received the vitamin-A-deficient ration were placed on range, the average blood phosphatase value decreased from 14.9 to 12.6 units within a week, and the phosphorus decreased from 6.5 mg./100cc. to 5.3 mg./100cc. Auchinachie and Emslie (1934) report a similar case in which a group of White Leghorn pullets which were receiving a cereal meat-meal "all mash" ration with access to oyster shell ad lib. consistently exhibited a much lower plasma phosphatase when they had access to sunshine than control groups which were kept inside even though the ration in the latter case was supplemented with from 250 to 20,000 units of vitamin D daily. Also of interest is the finding of these workers that the highest phosphatase values for the "sunshine" group did not occur at the time of minimum sunshine (January and February) but in the early summer.

CONCLUSIONS

1. The average plasma phosphatase values for five-month-old cockerels and pullets were 17.8 and 22.9 units respectively. These values were respectively 10 and 33 per cent higher than the average values obtained on whole

blood. Similar comparisons of sixteen-month-old birds showed the values for plasma to be lower than for whole blood, though the difference was not great.

2. Average plasma and whole blood phosphatase values for birds at five months were from 58 to 150 per cent higher than for birds of corresponding breed and sex of sixteen months.

3. There is an indication that the heavy breed hens (Rhode Island Red and White Wyandotte) may have higher phosphatase values than the lighter breed, White Leghorn.

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PART TWO. FLUCTUATIONS OF THE PHOSPHATASE AND INORGANIC PHOSPHORUS IN THE BLOOD OF THE LAYING HEN DURING THE PERIOD OF EGG FORMATION

INTRODUCTION AND REVIEW OF LITERATURE

Feinberg, Hughes, and Scott (1937) have recently reported that the blood serum of laying hens showed a marked rise in inorganic phosphorus during the period of shell formation. This increase is ascribed to the liberation of inorganic phosphate when calcium is drawn from the calcium phosphate of the bones to be laid down as calcium carbonate in the shell.

This view is substantiated by the findings of Deobold, Lease, Hart, and Halpin (1936) that about 10 per cent of the bone of hens on a liberal calcium ration is available for shell formation; and, further, by the work of Kyes and Potter (1934) in which it was demonstrated that pigeons in a laying condition showed ossification of the bone marrow while males and non-laying females did not.

Common (1932, 1933), as a result of his work with the excreta of hens, offered a similar explanation. He found a rise in the phosphorus content of the excreta correspond-

ing to the period of shell formation and attributed the excess phosphorus to phosphate set free when the calcium was removed from the bones for shell formation.

In view of the considerable body of evidence which has accumulated pointing to the specific biological role of phosphatase in the deposition and maintenance of the mineral salts present in bones, it would seem that a study of the fluctuations of plasma phosphorus and phosphatase at definite periods between ovulation and oviposition of the egg might possibly answer the question as to whether phosphatase is an active agent in shell formation. This question has been considered by Auchinachie and Emslie (1934) who have shown that in normal hens the serum phosphatase is not greatly affected by egg-laying, but in hens suffering from vitamin-D deficieney the value is increased during laying, and is perhaps due to the withdrawal of calcium from the bones. Common (1934, 1936) obtained results that were similar. He could not find a direct connection between serum phosphatase and intensity of egg production; but birds on a low calcium diet showed increased serum phosphatase on starting to lay, and the values remained higher than for the control during the course of the experiment.

Since previous reports indicate that the plasma phosphatase of laying hens varies widely, it was decided that a study designed to determine whether these variations might be associated with the stages of the cycle of egg formation might contribute to the solution of this problem. In this study, data on the phosphatase and phosphorus content of the plasma of laying and non-laying hens at the different stages are presented.

METHODS USED

Laying hens were bled at intervals during the cycle of egg formation, one hen being used for each complete set of bleedings. The intervals chosen were identical with those of Feinberg, Hughes, and Scott (1937). Three bleedings corresponding to various shell and non-shell depositing periods were made, and about five cubic centimeters of blood were collected at each bleeding. The method of drawing and handling the samples has been described previously[1]. Since three samples were taken from each bird, as great care as possible had to be taken to stop the flow

[1]Part One of this thesis.

of blood immediately after withdrawing the needle from the vein. The method described by Feinberg (1937) was found to work in most cases. Bleedings one and two were made from the same wing vein in cases where the flow of blood stopped without intraveinous clotting; if that happened, it was necessary to make the second and third bleedings from the other wing vein.

Period one (usually at 8:00-10:00 a. m.) represents the interval immediately following oviposition of the previous egg and the ovulation of the one under consideration. Period two, eleven and one-half to thirteen hours after oviposition, has been demonstrated by Warren and Scott (1935) to represent a time of rapid shell deposition. Period three was about twenty-six hours after oviposition of the previous egg, or about time for oviposition of the egg being studied.

In this work, period two was not determined, as was the case in the work of Feinberg, Hughes, and Scott (1937), by the detection of a soft shell egg in the uterus by digital cloacal manipulation. Instead, the birds were bled at the three intervals described and the time of second oviposition was verified. To determine the influence of any diurnal factor, control non-laying hens were bled at

approximately the same time as were the laying hens. Phosphatase and inorganic phosphorus were determined as has been described previously[2]. White Leghorn hens were used throughout. All birds were on normal laying rations with access to sunshine and green range, except the young seven-month-old pullets were kept inside for the course of experiment (about one week).

RESULTS

The data presented in Table 1 were obtained on sixteen-month-old hens that had been laying moderately well for nine months. The average plasma phosphatase value of the nine non-laying hens increased during the successive periods - the value for period two being 15 per cent higher than for the first period, and the value for period three being 8 per cent higher than for period two. The average phosphatase value for 12 laying hens was 30 per cent higher at the period of active shell deposition than at period one, falling off only slightly (5 per cent) just before the time for a second oviposition. The fact that phosphatase

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[2] Part One of this thesis.

Table 1. Average level of plasma phosphorus and phosphatase of 12 laying and 9 non-laying hens (age 16 months) at different stages of the cycle of egg formation.

Bleeding period*	Inorganic phosphorus (mg/100 cc.)		Phosphatase (Bodansky units)	
	Non-laying hens	Laying hens	Non-laying hens	Laying hens
1	4.7	4.0	8.4	5.9
2	4.3	4.6	9.7	7.7
3	4.5	4.4	10.5	7.3

*Period 1 - interval immediately following oviposition of the previous egg.
Period 2 - eleven and one-half to thirteen hours after oviposition of the previous egg.
Period 3 - about twenty-six hours after oviposition of the previous egg.

values were in all cases higher for period three than for period one is difficult to explain, especially in the case of the non-laying hens. There is the possibility that the frequent bleeding and handling were responsible.

The inorganic phosphorus levels were not significantly different for any of the three periods in the case of non-laying hens, but a slight increase was observed for the laying hens during the period of active shell formation. Thus, for periods two and three respectively the inorganic phosphorus level was found to be 15 and 10 per cent higher

than that found in the first period.

The investigator was unable to duplicate the findings of Feinberg, Hughes, and Scott (1937) who have reported that for laying hens the phosphorus level increased to 177.5 per cent during the period of active shell deposition. As a matter of fact, the variations in inorganic phosphorus during the cycle of egg formation as found in the present study for laying hens corresponds almost identically with that reported by Feinberg et al. for non-laying hens (113.5 and 111 per cent respectively for periods two and three). Since the present observations were made during the very warm summer months (July, August), it is possible that this discrepancy may have been due to temperature or seasonal factors.

A similar study of seven-month-old pullets in their first week of lay gave some interesting differences (Table 2). Also included are values for ten-month-old cockerels bled at the same time. The average plasma phosphatase value of 10 non-laying pullets, previous to coming into production, was seven per cent higher for period two than for the first period as compared with a 15 per cent increase for the older birds. The average value for laying hens, however was 35 per cent higher at the period of active shell dep-

Table 2. Average level of plasma phosphorus and phosphatase of 11 laying, 10 non-laying pullets and 3 cockerels at different stages of the cycle of egg formation.

Bleeding period*	Inorganic phosphorus (mg/100 cc.)			Phosphatase (Bodansky units)		
	Laying hens	Non-laying hens	Cockerels	Laying hens	Non-laying hens	Cockerels
1	5.6	4.8	3.7	12.6	15.6	7.6
2	7.8	4.1	3.7	17.0	16.6	8.1
3	6.1	4.7	3.4	13.9	14.1	6.9

*Same as Table 1.

osition then at period one. This was only a small increase over that observed in the case of the sixteen-month-old birds. Although the values for cocks are lower, the percentage differences are almost the same as those for non-laying hens.

The most significant difference observed between the two age groups was in the case of the plasma phosphorus of the laying hens. Thus, for periods two and three respectively, the inorganic phosphorus level of the pullets was 39 per cent and 9 per cent greater than that found in the first period, as compared with 15 per cent and 10 per cent for the older hens.

The plasma phosphatase activity of pullets which had just begun to lay was considerably higher than that of birds which had been laying for sometime. The further observation that the plasma phosphatase of non-laying hens was in most cases slightly higher than that of laying hens suggested a special study of these groups.

As shown in Table 3, the average plasma phosphatase of seven-month-old pullets which had not yet begun to lay was 14.0 as compared with 12.7 for birds of the same age which were laying. In view of the wide individual variation in both cases it is probable that the difference is not of great significance. However, somewhat greater importance might be attached to the values obtained on sixteen-month-old birds since the value for laying hens is 30 per cent lower than for non-laying hens. Auchinachie and Emslie (1934), in a similar study, observed no significant difference between the values for strictly normal laying and non-laying hens. While Common (1934, 1936) found that non-layers showed an average phosphatase value that was lower than for layers, he did not attach any significance to the results in the case of adult birds. It is interesting to note that for 32 adult White Wyandotte laying hens he found a greater range of values and a ten-

Table 3. Range of phosphorus and phosphatase in the blood plasma of laying and non-laying hens.

Group	No. in group	Inorg. phosphorus (mg/100 cc.) Range	Inorg. phosphorus (mg/100 cc.) Average	Phosphatase (Bodansky units) Range	Phosphatase (Bodansky units) Average
Non-laying hens (7 months)	14	3.7-6.4	4.6	5.1-27.6	14.0
Laying hens (7 months)	16	3.0-7.8	5.2	4.0-30.2	12.7
Non-laying hens (16 months)	9	2.9-6.2	4.7	2.8-20.4	8.4
Laying hens (16 months)	27	3.1-5.9	4.5	2.9-14.0	5.9

dency for the values to be higher than in the case of the present study on White Leghorns (Table 4).

Table 4. Distribution of phosphatase values (Bodanksy units).

Twenty-seven White Leghorn hens
(16 months old)

Range:	0-4	4-8	8-12	12-16	16-20	20-24	24-28	28-32	32-36	36-40	40-44
No.	4	19	3	1	0	0	0	0	0	0	0

Thirty-two White Wyandotte hens*

Range:	0-4	4-8	8-12	12-16	16-20	20-24	24-28	28-32	32-36	36-40	40-44
No.	1	3	10	4	4	2	2	4	0	1	1

*Common (1934, 1936).

DISCUSSION

It is interesting that the variations in the phosphatase during the different stages of the egg cycle parallel the variations in the values for inorganic phosphorus. How much significance can be attached to the increase in phosphatase during the period of active shell deposition in the case of the older birds might be questioned on the basis that the values for the control non-laying hens also increased during this period though not as markedly. If the non-laying hens are reliable measures of the uncontrolled causes of variation to which the laying hens were also exposed, then the validity of the values is more clearly brought out by expressing the plasma phosphatase activity of laying hens in terms of its percentage of the plasma phosphatase value of non-laying hens. Thus for periods one, two, and three respectively the values of 70, 80, and 70 per cent are obtained. A similar comparison on the percentage basis in the case of inorganic phosphorus does not impair the relationship of the real values for inorganic phosphorus already pointed out. The values of 85.1, 107.0, and 98 per cent are obtained. The difference

in the phosphatase levels for periods one and three are difficult to explain, especially since these periods are practically identical in the cycle of egg formation. It is of interest that a study of the three periods in the case of cockerels shows the phosphorus does not vary significantly, but on a percentage basis the phosphatase variations are about the same as found for non-laying hens.

As has previously been pointed out (Feinberg, Hughes, and Scott (1937); (Deobold, Lease, Hart, and Halpin (1936); (Kyes and Potter (1934), the hen has available a ready reserve from which she can draw calcium as rapidly as it is being deposited in the shell. As the calcium is drawn from the calcium phosphate of the bones inorganic phosphate is liberated and manifests itself in the increased inorganic phosphorus level of the blood.

No doubt the amount of calcium being absorbed from the digestive tract at the time of shell deposition would have an effect on the amount of calcium withdrawn from the bone for shell formation. This would influence the amount of phosphorus liberated at that time. At no time during the present study was the calcium carbonate content of the digestive tract known.

The significance of increased phosphatase is not so

easily explained. It may be that the enzyme plays an active part in the demineralization of bone. Common (1936), in studying this question, found that pullets on a low calcium diet showed greatly increased phosphatase levels in the blood and phosphorus excretion immediately on starting to lay. It has been suggested by Kay (1932) that phosphatase may synthesize soluble phosphoric esters of calcium from some of the insoluble calcium phosphate of the bone. Again he suggests that the enzyme may be concerned with the depositing of the phosphate in the bone. If this is the case, we would not expect the phosphatase level during shell deposition to be greatly higher than at other times of the day since the calcium phosphate removed from the bone would need to be restored, at least in part, if the hen is to remain normal.

The possibility of phosphatase being an active agent in shell formation has been considered by Auchinachie and Emslie (1934) from a different viewpoint. From studies on the distribution of phosphatase in the oviduct and ovary these workers have concluded that inasmuch as the phosphatase activity of the shell gland proved to be very low the enzyme must play, at the most, only a minor part in shell formation. Their studies of this phase of the

problem, however, did not include a study of the changes that occur during the cycle of egg formation.

CONCLUSIONS

1. The average plasma phosphatase value for 12 laying hens which had been laying moderately well for nine months was 30 per cent higher at the time of active shell deposition than at the start of the twenty-six-hour period of the egg cycle. Analysis of the blood plasma for inorganic phosphorus during a similar period revealed a 15 per cent rise in the phosphorus level.

2. The average phosphatase value for 11 seven-month-old pullets in their first week of lay was 35 per cent higher at the period of active shell deposition than at the start of the twenty-six-hour period of the egg cycle. During the same period, plasma inorganic phosphorus rose 39 per cent.

3. Cockerels show relatively constant phosphorus values, and phosphatase values lower and variations of the same order as non-laying hens for the three periods.

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ACKNOWLEDGMENTS

The author wishes to take this opportunity to express his appreciation to Dr. J. S. Hughes and Dr. W. J. Peterson for assistance in selecting this problem, and especially to the latter whose constant encouragement and assistance contributed materially to the carrying on of this work. He is also indebted to Prof. L. F. Payne and Dr. D. C. Warren of the department of Poultry Husbandry for the loan of experimental birds used in this study. To fellow students and members of the staff at the College Poultry Farm, he is indebted for assistance at various times while carrying on this work.