CCLXII. THE SIGNIFICANCE OF PHOSPHATASE ESTIMATIONS IN THE ADULT FOWL.

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Kay [1930] showed that in certain bone diseases in man the plasma-phosphatase activity is considerably higher than normal. This served as an impetus for investigation into the activity of the enzyme in plasma and tissues under different conditions in man and experimental animals. The literature has been reviewed by Kay [1932] and by Robison [1932], and it is unnecessary to go into it in great detail here. Briefly, it may be said that in healthy young animals the activity of the plasma-phosphatase is higher than it is in the adult, but with the completion of rapid bone formation the value drops to the adult level. This is well illustrated by the observations of Stearns and Warweg [1933] with children. During the healing of rickets or other bone diseases the initially high plasma-enzyme activity tends to return to the normal for animals of that age and class. Abnormally high values may be observed in jaundice [Roberts, 1933] and possibly also after the ingestion of a high carbohydrate meal [Bodansky, 1934]. When proper precautions are taken it would seem that estimation of the plasma-phosphatase activity may serve in many mammals as a somewhat earlier indication of faulty Ca and P metabolism than do serum-Ca or inorganic P determinations, although high phosphatase values cannot be considered specific for any one disease.

Clinically, estimations of tissue-phosphatase activity have a more limited value. Bone-phosphatase activity is high in rickets: it is diminished under prophylactic, and still more under excessive, treatment with vitamin D. Hypervitaminosis D also results in a decrease in the activity of the kidney-phosphatase. It has been shown that the tissue-phosphatase of fowls reacts in a manner similar to that of mammals [Hall and King, 1930–31; King and Hall, 1931].

Within the species, the individual variation of the plasma-phosphatase activity of normal adult mammals is not great [Kay, 1930; Auchinachie and Emslie, 1933]. From the values reported by Common [1934] it would appear that the normal range is wide in adult fowls. From the three values he gives for cockerels one might consider the range in these birds to be narrow, but the laying pullets vary from 6·9 to 27·7 Bodansky [1933] units, or approximately 0·23–0·92 Kay [1930] units[1]. These birds were considered to be strictly normal White Wyandotte pullets (personal communication) and the question naturally arises as to whether estimations of the plasma- (or serum-) phosphatase activity will give any indication of faulty Ca and P metabolism in fowls, or whether there is normally such variation that phosphatase estimations have only a limited significance at the present time.

[1] Assuming one Kay unit to be equivalent to approximately 30 Bodansky units.

Referring to Common's figures it can be seen that the nine values for sexually immature pullets ranged only from 1·7 to 5·3 units (approximately 0·06–0·18 Kay units) and that for the pullet nearing laying was 12·1 units. It may seem that not only during, but also in preparation for, laying there is an increase in the serum-phosphatase activity. Common [1932; 1933] had previously shown by means of "balance" experiments that during the pre-laying period there was an increase in the percentage retention of Ca. During the laying period there were large amounts of P excreted, which he related to shell formation. The theory was that if, as apparently occurred under the conditions of his experiments, there was too little Ca in the food for the proper formation of the shell, the bird was able to draw on Ca reserves in her body. Such reserves were located, presumably, in the bones: withdrawal of CaO for shell formation necessitated the excretion of equivalent amounts of P_2O_5 resulting from the breakdown of $Ca_3(PO_4)_2$. This theory can probably not be applied *in toto* to account for the variations in the serum-phosphatase of the laying pullets mentioned above. They had access to good grass runs and to a Ca supplement *ad lib.*, and the values for the two moulting pullets were 13·9 and 24·0 units. There is the possibility, of course, that the blood samples were taken during a period of dull weather, and that no vitamin D supplement was provided in the food.

Lest investigators should conclude that estimations of plasma-phosphatase activity of fowls are valueless owing to the wide and unexplainable variations likely to be encountered, we thought it might be of interest to record the results of some of our observations. We commenced our observations in 1931, and while our data are by no means complete, several interesting points regarding the interpretation of plasma-phosphatase estimations in adult fowls have arisen.

Methods.

For plasma-phosphatase, the procedure of Kay [1930] was used. Certain modifications (chiefly in dilution of plasma) were necessary when working with very active samples, but all results were calculated on mg. P liberated by 1 ml. plasma under the conditions otherwise defined by Kay. Tissue-phosphatase was estimated by the method described by Kay [1928]. The Clark-Collip [1925] modification of the Kramer-Tisdall method, and the Havard-Reay [1925] adaptation of the Briggs method were used for serum-Ca and blood-inorganic P respectively.

Blood was drawn from the wing vein through a paraffined needle direct into the centrifuge-tube. All determinations were made in duplicate on blood samples of individual birds: the blood was not "pooled." It might be mentioned that no significant difference was found when comparisons were made of the phosphatase activity of oxalated blood-plasma, "native" plasma and serum. The average "paradoxical" increase [Bodansky, 1933] in the phosphatase activity of 14 samples of plasma, stoppered and left at room temperature for 19 hours, was 0·01 unit.

Results.

We have found the values for strictly normal mature birds to vary only slightly, as shown by the data in Table I.

There is very little individual variation in the values obtained for the cockerels, especially after they have reached maturity. The pullets show a somewhat wider range, and this is especially noticeable in the W.W. pullets. There may be a breed difference but it is probably insignificant and accounted for by the greater

Table I. *Plasma-phosphatase of normal fowls.*

	No.	Age months	Units plasma-phosphatase Average	Range	Date
R.I.R. cocks	5	7	0·32	0·28–0·38	16. vii. 32
,, ,,	9	8	0·15	0·12–0·20	19. viii. 32
,, ,,	9	9	0·11	0·05–0·18	23. ix. 32
,, ,,	7	11	0·14	0·10–0·18	26. ix. 32
,, ,,	6	13	0·16	0·14–0·19	16. i. 33
R.I.R. pullets	6	15	0·22	0·14–0·34	18. vii. 32
W.W. ,,	34	17	0·31	0·14–0·57	17. ix. 32
W.L. ,,	16	15	0·14	0·09–0·20	18. vii. 32

Note. The following abbreviations are used in this paper: R.I.R. = Rhode Island Red; W.W. = White Wyandotte; W.L. = White Leghorn.

variation in the W.W. pullets. All the birds mentioned in Table I were receiving a good commercial ration and had free access to grass runs and oyster shell grit. From outward appearances they were quite normal: there was no significant difference in the blood-inorganic P, and the serum-Ca showed the normal range for hens. Further investigation into factors affecting plasma-phosphatase activity may indicate why the individual variation appears to be so much greater among W.W. than among R.I.R. or W.L. pullets.

Several factors may account for individual variations in the units of plasma-phosphatase activity of birds which appear comparable in other respects. Bodansky [1934] considered that differences might occur due to "increased functional activity of the organs and tissues involved in the processes that follow ingestion." The diet given to fowls is rich in carbohydrate. The plasma is often opalescent, although there may be no definite lipaemia. We were unable to find any significant difference in the plasma-phosphatase activities of chickens which were given saline, starch or egg-white by stomach tube. Samples were taken 1·5–4·5 hours after feeding. The chickens weighed 100–200 g. They were starved 24 hours and given 2·5 g. of starch or egg-white suspensions. The controls received the same volume of saline. In experiments with adult fowls, no attempt was made to draw blood samples when the birds were in a post-absorptive state and, apart from the W.W. pullets mentioned in Table I, the range of values for normal birds was not high. This is especially so in the case of the cocks, where there is no question of differences due to egg laying.

Bodansky [1934] has confirmed his earlier finding that fasting results in a lowering of the serum-phosphatase of dogs. It appears that this probably occurs in fowls also. W.L. cockerels were starved for periods up to 168 hours and blood samples taken at 24-hourly intervals. The average values for the different hours are shown in Fig. 1. It will be noticed that the initial value is high. This is because the birds were between 3 and 4 months old. The value at 168 hours is similar to those we have observed in adult cocks. Whether starvation of the latter would result in as noticeable a decrease in the phosphatase activity is doubtful, but there would probably be some decrease. It was found difficult to assess the effect of fasting on the activity of the tissue-phosphatase because of the wide individual variations. It appeared that there might be a general downward trend in the activity of the bone-enzyme. This was certainly not the case with the phosphatase of either the kidney or the intestinal mucosa.

The values for the pullets in Table I are somewhat higher than those for the mature cocks. Some of the pullets were laying. The question naturally arises, does egg formation under normal conditions of itself cause an increase in the

plasma-phosphatase activity? A hen may lay on each of 26 consecutive days and may lay 300 eggs a year. An egg weighing 56 g. contains approximately 2 g. Ca and 0·1 g. P. Almost all the Ca is in the shell. Pearl and Surface [1912] estimated that shell formation was completed in 12–16 hours [*cf.* Asmundson, 1930–31]. It can readily be seen that, compared with bone calcification, the laying down of the egg-shell is an extremely rapid process, and phosphatase might be expected to play as large a part in the latter as it is commonly thought

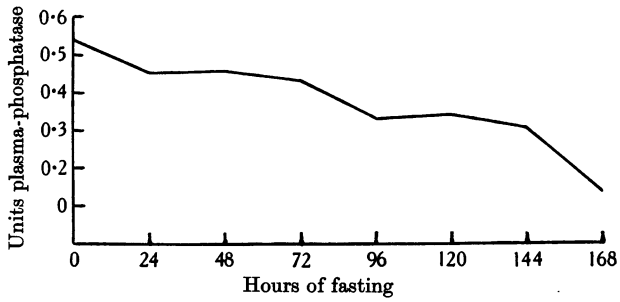

Fig. 1. Plasma-phosphatase of W.L. cockerels during fasting.

to do in the former. Heller *et al.* [1934], indeed, appear to believe the two processes to be identical. The similarity, however, is far more apparent than real: to mention only one thing, the normal egg-shell contains some 97 % $CaCO_3$. Halnan [1925] concluded from his experiments that there was a relatively short latent period between the absorption of Ca from the food and its excretion as egg-shell. McGowan [1934] believes that Ca is absorbed from the alimentary canal as $CaCl_2$ and circulates as such and in combination with protein. In rabbits fed a $CaCO_3$-rich diet, $CaCO_3$ is excreted by the kidneys, whereas in the laying hen $CaCO_3$ is excreted almost wholly by the shell gland. Utilisation of food Ca for shell formation decreases the precipitation of calcium phosphate in the tissues and decreases the P retention. That Common was unable to agree with Halnan was possibly due in part to the less abundant supply of Ca in the diet of his pullets. It is inconceivable that a heavily laying hen can continue for long to draw on her body reserves of Ca for shell formation. That she is able to do so for a short time under certain conditions, however, is incontestable.

While admitting that there seems little reason to believe that under optimum conditions there should be more "leakage" of phosphatase from the bones of a laying hen as compared with one not laying, there is still the possibility that phosphatase is an active agent in egg formation, and that the enzyme may enter the blood-stream sporadically from the ovarian and oviducal tissue. The distribution of phosphatase in the oviduct and in the ovary is shown in Table II.

Table II. *Phosphatase in oviduct and ovary.*

Oviduct.	Shell gland	trace.
	Isthmus	trace.
	Albumin-secreting portion ...	trace to about 0·3 unit.
Ovary.	Large ova	trace.
	Small white ovules	2·0–6·0 units.
	Ovary	about 3·0 units.

The phosphatase activity of the shell gland of the oviduct is very low and, while evidence of this kind is by no means conclusive, it at least indicates that the

enzyme plays at the most only a very minor part in shell formation. There is a definite phosphatase activity in the small "white" ovules, and it would appear that the enzyme has an important rôle in the primary stages of yolk formation. This is a more or less continuous process, and there should be no great individual variation in the plasma-phosphatase activity of strictly normal hens which are laying with approximately the same intensity. Nevertheless it is possible that, owing to "leakage" of the enzyme, the plasma-phosphatase activity of laying hens may be higher than that of non-layers. We have not, however, observed any significant difference between the values for strictly normal laying and non-laying hens (Table III).

Table III. *Plasma-phosphatase of normal laying and non-laying hens.*

Breed	Condition	No.	Units phosphatase	
			Average	Range
W.W.	Laying	27	0·32	0·14–0·50
W.W.	Non-laying	7	0·35	0·29–0·57
W.L.	Laying	7	0·14	0·09–0·20
W.L.	Non-laying	9	0·14	0·10–0·20

Under sub-optimum conditions, however, such as might be expected to occur when there is an inadequate amount of vitamin D available, it should not be surprising to find higher values for plasma-phosphatase in laying as compared with non-laying birds. This is because, (*a*) the vitamin D requirement of the non-laying hens and cocks is presumably lower than that of the laying hens, and so their vitamin reserves should be depleted less rapidly, and (*b*) owing to faulty Ca absorption or utilisation brought about by (*a*), the laying hens may draw on their body reserves of Ca for shell formation and the plasma-phosphatase activity may increase for the same reason that it does in osteoporosis in mammals. This is illustrated by the following data obtained with W.L. pullets kept indoors for 2 months and fed a cereal protein-supplemented mash and oyster shell *ad lib.*, but with no additional vitamin D.

No. of eggs laid	0	1–5	6–50
Av. units plasma-phosphatase	0·30	0·48	1·21
Standard error	0·019	0·086	0·147
No. of birds	19	5	24

In the calculation of these averages, the values obtained with one pullet have been excluded. The activity of her plasma-phosphatase was consistently low, *e.g.* 0·3 unit when she had laid 30 eggs. She subsequently died, and on *post mortem* examination multiple tumours were found throughout the peritoneum. Whether this has any bearing on the low phosphatase values we are not in a position to say.

The serum-Ca of laying hens may vary within wide limits even when inadequate vitamin D is supplied. The blood-inorganic P remains at a fairly constant level. When experiments are planned for investigating the Ca and P metabolism of adult fowls, data of serum-Ca and blood-inorganic P determinations often have only a limited significance. It is possible that estimations of plasma-phosphatase activity may prove of value in such cases. The interpretation of the data, however, requires a consideration of a number of factors.

It would appear that in the adult fowl, at least, temporary increases in the plasma-phosphatase activity due to alimentation are little likely to obscure other differences, and it should be easy enough to allow for the effect of fasting. It has been shown that continuance of egg production when inadequate vitamin D

is supplied may result in an increase in the plasma-phosphatase activity. Theoretically, this may also occur when the Ca supply is inadequate. In both cases there is an upset in the Ca and P metabolism. If the view be taken that vitamin D assists in the absorption and utilisation of Ca, then in hypovitaminosis D, as in hypocalcicosis, the formation of normal shells may be expected to lead to some degree of osteoporosis. Common's theory has already been mentioned. It is not unlikely that had determinations been made of the plasma-phosphatase activity of his "balance" experiment pullets, values higher than normal might have been found. Osteoporosis is not a very rare condition to find in laying birds: two such cases we examined had plasma-phosphatase values of 12 and 15 units respectively.

It is not sufficient to conclude, however, that because a group of hens is kept on a diet which contains at the most only small amounts of vitamin D that an increase will necessarily be found in the phosphatase activity of the plasma of each bird. *Per contra*, other groups may be kept on diets containing 1 % of cod-liver oil and with access to oyster shell *ad lib.*; or with 1 % of cod-liver oil and 5 % of $CaCO_3$ in the diet, and increases in the phosphatase activity of the plasma of some of the birds may still occur. Wide individual variations in hens kept under these conditions may be expected. This is easily understood if one considers the difference there may be in the intensity of laying, the size of egg laid, the size of bird and other less easily measured factors such as body reserve of vitamin D or of Ca and minimum requirement for vitamin D or Ca. A diet may be inadequate in vitamin D for one bird and yet adequate for another. The same may occur with regard to Ca.

Two experiments may be mentioned to illustrate the variations in the activity of the plasma-phosphatase which may occur. In one experiment, W.L. pullets were fed different amounts of vitamin D but had access to oyster shell *ad lib.* Six groups of pullets were kept indoors and fed a cereal—meat-meal "all mash" ration. One group received no additional vitamin D, four groups received varying amounts of radiostol[1], and one group received 1 ml. of cod-liver oil per bird daily. Both the radiostol and cod-liver oil were given orally. Another group was fed the same "all mash" ration as the indoor groups but was provided with an outside board-covered run. Determinations of serum-Ca, blood-inorganic

Table IV. *Plasma-phosphatase of W.L. pullets.*

Group and supplement		Range in plasma-phosphatase values					
		Jan.	Feb.	Apr.	May	June	July
A. None (indoors)	Lowest	0·22	0·28	1·85	0·85	0·68	0·25
	Highest	1·22	2·22	2·96	3·97	3·97	3·93
B. 1 drop radiostol	Lowest	0·44	0·26	0·31	2·46	2·63	1·65
	Highest	0·76	1·26	3·21	3·95	5·21	3·89
C. 7 drops ,,	Lowest	0·35	0·11	0·49	3·16	5·91	0·88
	Highest	1·72	1·31	5·85	6·96	5·97	6·03
E. 1 ml. ,,	Lowest	—	0·21	1·00	0·27	0·22	0·27
	Highest	—	1·32	6·94	3·70	5·96	4·14
F. 2 ml. ,,	Lowest	—	0·07	0·09	0·07	0·40	0·25
	Highest	—	1·27	6·41	4·07	5·95	5·61
G. 1 ml. cod-liver oil	Lowest	—	0·18	0·22	2·32	3·12	1·38
	Highest	—	1·19	5·56	3·94	3·97	6·02
D. None (outdoors)	Lowest	0·18	0·26	0·40	0·63	0·48	0·23
	Highest	0·35	0·91	1·03	1·50	1·60	1·65

[1] Radiostol (B.D.H.) 10,000 units vitamin D/ml. One drop equivalent to approximately 250 units vitamin D.

P and plasma-phosphatase were made at approximately monthly intervals. There was no significant change in the blood-inorganic P, and the serum-Ca values showed the normal range for mature hens. There was a wide individual variation in the plasma-phosphatase activity. This is illustrated in Table IV, in which the range only of the plasma-phosphatase values of the different groups is given.

There seemed to be no relation between the vitamin content of the diet and the activity of the plasma-phosphatase. No one group can be said to be significantly worse than another. There was no "leg weakness" in group A. Birds in groups A–C inclusive ceased to lay. Conditions did not permit of trap-nesting the birds, and it may be that the very wide individual variations are partly explainable on differences in size of egg and intensity of egg production. Owing to the small number (7) of hens in each group and to the differences in the mortality it is not possible to compare the egg production, and it cannot legitimately be said that as many as 7 drops of radiostol (group C) were less effective as a vitamin D supplement than 1 ml. of cod-liver oil (group G). It is noticeable that the values for group D (which had access to sunshine) were not so high as was common for the birds kept inside. On the other hand, the peak in the values for this group did not occur at the time of minimum sunshine.

In another experiment, R.I.R. pullets were kept indoors and fed a cereal-fish-meal ration containing 1 % cod-liver oil and 5 % $CaCO_3$. No oyster shell was supplied. The birds were trap-nested. Blood samples were drawn at approximately monthly intervals. The results of plasma-phosphatase estimations on two such months are given in Table V, together with the total number of eggs laid by each bird up to that date.

Table V. *Number of eggs and plasma-phosphatase. R.I.R. pullets.*

	16. viii. 32		23. ix. 32	
Pullet No.	No. of eggs	Units phosphatase	No. of eggs	Units phosphatase
88	13	0·67	38	0·63
85	17	0·51	35	0·69
82	18	0·56	39	2·48
84	18	0·25	44	0·74
87	18	0·42	45	0·43
86	21	0·61	50	0·44
79	22	0·73	47	0·92
80	22	0·24	38	0·23
83	24	0·67	40	0·74
77	25	0·92	49	3·50
81	25	0·23	54	0·25
78	26	0·37	37	1·41

So far as one can judge from the data, increase in the plasma-phosphatase values was not related to intensity of laying alone. It might appear that while the diet was adequate for some of the birds, it was not for others. Analyses of the eggs for Ca content, *etc.*, would probably have thrown some light on the results. On the other hand, some experiments have indicated that when two pullets are fed the same diet, one bird may be in Ca equilibrium (*i.e.* Ca in food = Ca in excreta + eggs) and produce eggs with progressively decreasing content of Ca, while the other may be on a negative Ca balance (*i.e.* drawing on her body reserves of Ca for shell formation) with little difference in the amount of Ca in her eggs. It would seem, therefore, that for estimations of plasma-phosphatase to be of any real value in experimental work with adult fowls, it is

necessary to bear in mind the possible effect of many different factors, only one of which is the number of eggs laid.

While this is so, there is nevertheless more than a mere suggestion that an abnormally high plasma-phosphatase activity does indicate that conditions are not optimum for the Ca and P metabolism of that bird. Should any question arise as to whether a certain group of birds may be benefited by the administration of vitamin D, for example, plasma-phosphatase estimations may give an indication. It is considered by many poultrymen that in Great Britain it is unnecessary to give cod-liver oil to fowls which have access to outdoor grass runs. Oyster shell is provided *ad lib.* We had occasion in March, 1933, to examine a number of pullets which were kept under such conditions. They had severe "leg weakness." With two exceptions the plasma-phosphatase activity was abnormally high. The birds were given approximately 1 ml. cod-liver oil per bird daily, and in ten days there was no sign of the "leg weakness." Blood-samples were taken the following month. The data are given in Table VI.

Table VI. *Plasma-phosphatase of "leg-weak" hens.*

	Units	
Hen No.	9. iii. 33	15. iv. 33
88	0·36	0·40
14	0·78	0·35
92	1·06	0·53
21	2·65	0·27
26	3·25	0·98
38	3·59	0·29
67	5·64	0·38
39	6·01	0·20
28	9·58	0·32
35	11·84	0·69
47	15·09	1·39

It should be explained that the weather had been rather dull during the latter part of February. Inactive birds tend to remain in the laying house more than do the active and often more heavily laying hens. How much the lower phosphatase values of hens Nos. 88 and 14 were due to the "leg weakness" being so severe as to result in an actual starvation it is difficult to say, but it seems not improbable.

It might be mentioned in this connection that the feeding of toxic amounts of vitamin D may lead to a slight decrease in the plasma-phosphatase activity. This may be accounted for both by the actual effect on the bone-phosphatase and by the anorexia which also occurs. The data obtained with two cocks are presented in Table VII. These birds were given 500,000 units of vitamin D (1 ml. calciferol-olive oil solution) daily for ten days.

Table VII. *Effect of 500,000 units vitamin D daily. R.I.R. cocks.*

Cock No. 223	24. xii. 33	29. xii. 33	3. i. 34
Serum-Ca (mg./100 ml.)	12·45	17·10	18·25
Inorganic P (mg./100 ml. blood)	3·99	4·08	3·07
Plasma-phosphatase (units)	0·34	0·14	0·10
Body weight (kg.)	2·36	2·24	2·05
Cock No. 260			
Serum-Ca (mg./100 ml.)	12·15	18·30	24·30
Inorganic P (mg./100 ml. blood)	4·08	4·99	3·23
Plasma-phosphatase (units)	0·31	0·14	0·09
Body weight (kg.)	2·09	1·77	1·85

Anorexia and extreme thirst occurred after the fourth day. There was a definite increase in the serum-Ca, but little change in the blood-inorganic P. Laying pullets have been found to be far more resistant to the toxic effect of large doses of calciferol (500,000 units vitamin D daily for 7 days) than is the case with non-laying pullets, which react in a similar manner to the cocks. This point seems of significance in studying the fundamental action of vitamin D, and the mode of formation of the egg-shell.

In conclusion it is suggested that estimations of plasma- and tissue-phosphatase activity may prove of value to those interested in the Ca and P metabolism of fowls. Mention of apparently anomalous results which may be obtained will not, it is hoped, deter investigators.

SUMMARY.

The significance of phosphatase estimations in a study of the Ca and P metabolism of the adult fowl has been discussed. It is suggested that the plasma-phosphatase activity of strictly normal hens may be little affected by egg laying, but that marked changes may be expected if the conditions are not optimum, such as may occur in vitamin D or Ca inadequacy. An attempt is made to explain why expected changes in the plasma-phosphatase activity may not always be obtained.

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