26. OBSERVATIONS ON THE SERUM PHOSPHATASE OF CATTLE AND SHEEP

By WILLIAM MILLER ALLCROFT AND
SYDNEY JOHN FOLLEY

*From the Ministry of Agriculture Veterinary Laboratory, Weybridge, and the
National Institute for Research in Dairying, University of Reading*

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THE presence in blood plasma or serum of an enzyme capable of hydrolysing esters of phosphoric acid optimally at an alkaline reaction, has been known for many years [see McCollum & Hart, 1908], and it is now evident that this phosphatase is the phosphomonoesterase A_1 in the classification of Folley & Kay [1936, 1]. Thus, for example, Gutman & Gutman [1940] state that normal human blood serum contains very little 'acid' phosphatase. The amount of phosphatase present in blood is considerably less than in such tissues as intestinal mucosa, bone epiphysis, kidney and mammary gland [see Folley & Kay, 1936, 1]. Normal values of serum phosphatase in arbitrary units have been given for man and certain animals by Kay [1930, 1] and for man and dog by King & Armstrong [1934], but as yet only limited data have been presented for the activity of this enzyme in the blood of farm animals.

Serum phosphatase is raised above normal in certain generalized bone disorders in man, such as rickets (infantile, renal and adult), generalized osteitis fibrosa, osteitis deformans and obstructive jaundice [see Folley & Kay, 1936, 1], and it has also been claimed that it is raised in certain other diseases such as tuberculosis and carcinoma, but the evidence here is less conclusive. Since serum phosphatase values have proved of diagnostic value in disordered Ca and P metabolism in man it was hoped that the same might hold in farm animals, as was suggested, on the basis of a limited number of observations on sheep, by Auchinachie & Emslie [1933].

Before the possible diagnostic value of phosphatase determinations in farm animals could be assessed it was obviously essential to know the limits of probable variation in the normal animal. This paper records data obtained for normal cattle and sheep at the National Institute for Research in Dairying, Shinfield, and the Veterinary Laboratory, Weybridge, these institutions maintaining close co-operation throughout the work.

The data for cattle were in the first instance obtained at Shinfield during the years 1933-5 in the course of investigations into the possible relationship between blood and milk phosphatases [see Folley & Kay, 1936, 2] and were later supplemented by data obtained on other herds to see if certain peculiarities characteristic of the Shinfield cows were also shown under different conditions of herd management and by other breeds, and by some data from Weybridge.

During the period 1934-5 investigations which included serum phosphatase determinations were made at Weybridge into 'cripples' of lambs, a disease described by Lyle Stewart [1933] upon which extensive biochemical studies

were made by Piercy [1934, 1, 2]. It was hoped that serum phosphatase estimations would aid diagnosis of the bone disorder for which there is evidence in this disease, but the data showed wide variations uncorrelated with the incidence of 'cripples'. A large-scale experiment on the aetiology of 'pining' in Northumberland sheep which was jointly conducted by Mr W. Lyle Stewart and Dr A. P. Ponsford of Newcastle-upon-Tyne and Dr H. H. Green and Dr E. L. Taylor of Weybridge gave an opportunity of acquiring an additional large number of serum samples for phosphatase determinations. 'Pining' is a loose term for a wasting disease of sheep associated in Northumberland with helminth infection and mineral deficiency, possibly of cobalt. In all 240 ewes and lambs were bled, some of them several times, and over 900 samples were available for blood studies in various directions.

It should be stated here that these sheep were apparently normal and showed no evidence of 'pining' over the period in which the phosphatase data were accumulated. These data were supplemented by a further 220 samples from normal ewes in other flocks.

EXPERIMENTAL

All blood samples were obtained from the jugular vein, with the aid of partial stasis caused by a choke-rope in the case of cattle and by thumb pressure in the case of sheep.

Serum phosphatase was determined in both laboratories by the method of King & Armstrong [1934] which involves incubation for 30 min. at 37°. Two slight modifications were made: (a) sufficient phenol was added to the phenol reagent used for blank determinations to give a colour readily readable in the ordinary colorimeter, and (b) before making the colorimetric reading all tubes were immersed in the water bath at 37° for 45 min. to develop the blue colour.

Blood samples taken near either laboratory were left until the next day to ensure complete coagulation and the sera were then analysed. Samples taken at distant centres were sent by rail in special ice-boxes. When a batch of samples was too large to be dealt with in one day, the sera not analysed on the first day were kept for one or, on some occasions, two days in the refrigerator. It was previously found by one of us (W. M. A.) that storage of serum in the refrigerator at 2° for periods up to 4 days made no appreciable difference to its serum phosphatase activity. All haemolysed sera were discarded as unfit for phosphatase determination.

RESULTS

The joint data are considered from a number of aspects, and for simplicity and economy of space the main features are presented in the form of distribution tables. In compiling these tables the values have been classified into groups covering 5 units for values from 0 to 30 units, 10 units for values between 30 and 50 units and thereafter in larger groups according to the data available. While all values were originally recorded to the first place of decimals, in the distribution tables they are, for simplicity, given to the nearest integer. Throughout this paper the term phosphatase refers to the phosphomonoesterase A_1 [Folley & Kay, 1936, 1] and all values are recorded in King & Armstrong units per 100 ml.

It will be noted that the number of samples exceeds the number of animals in the case of both cattle and sheep. This is due to the fact that 22 of the Dairy Shorthorn cows from one herd and 48 of the sheep from the 'pining' experiment at Newcastle-upon-Tyne were bled on several occasions. All the values so obtained have been included in the analysis of variability of results, since we

consider that this affords a better criterion of the extreme range of variation to be met with in normal animals. The alternative of entering the mean of the several values for each such animal in the distribution tables was considered and rejected, since this would tend to mask the extreme limits of variation. Only for investigation of the possible association between milk yield and serum phosphatase has the average value for each cow been taken.

Range of serum phosphatase in the apparently normal cow and ewe

For this study there were available 367 samples from 294 cows in 8 different herds maintained under varying systems of first-class dairy management, and 119 samples from the same number of Hereford cows kept under range conditions all the year round with the bare minimum of supplementary feeding in winter. For the ewe there are 555 samples from a number of pure-bred and cross-bred flocks. The overall ranges of values were 0.3–114.3 units for cows and 3.0–166.1 units for ewes. The summarized distribution data are given in Table 1.

Table 1. *Distribution of serum phosphatase values, actual and on percentage basis, in normal cows and ewes*

Serum phosphatase units	Dairy breeds		Herefords		All cows		All ewes	
	No.	%	No.	%	No.	%	No.	%
0.0–4.9	59	16.1	38	31.9	97	20.4	11	2.0
5.0–9.9	117	31.9	60	50.4	177	36.4	139	25.1
10.0–14.9	60	16.3	12	10.1	72	14.8	146	26.2
15.0–19.9	27	7.4	1	0.8	28	5.8	66	11.9
20.0–24.9	22	6.0	3	2.5	25	5.1	70	12.6
25.0–29.9	17	4.6	3	2.5	20	4.1	37	6.7
30.0–39.9	20	5.4	2	1.7	22	4.5	40	7.2
40.0–49.9	20	5.4	—	—	20	4.1	28	5.0
50.0–99.9	22	6.0	—	—	22	4.5	12	2.2
100.0 and over	3	0.8	—	—	3	0.6	6	1.1
Total no.	367		119		486		555	
Below 15 units		64.3		92.4		71.2		53.3
Below 40 units		87.7		100.0		90.7		91.7

The first thing to be noted in Table 1 is that, despite the wide overall range in each species, the greater proportion of values for both cows and ewes are under 40 units, though a small proportion of apparently normal animals show values above this limit, even exceeding 100 units in about 1 % of cases. Among the cows it will be seen that all values for the Hereford breed are below 40 units whereas there are 12.3 % of values over 40 units among the dairy breeds. Further discussion of this fact is left until later when possible breed differences will be considered.

Whereas in the data for cows 71.2 % of values are below 15 units and 19.5 % between 15 and 40 units, the corresponding figures for ewes are 53.3 and 38.4 % respectively. Considering now the values under 10 units, these are 56.8 % for cows and 27.1 % for ewes. These figures show that the scatter of values below 40 units is greater in ewes than in cows and suggests a species difference with the median value for ewes higher than that for cows.

The wide variations in apparently normal ewes and cows, particularly of the Dairy Shorthorn breed, revealed by the data in Table 1 make it evident that the diagnostic value of determinations of serum phosphatase in cases of suspected disorders of Ca and P metabolism in farm ruminants is likely to be extremely limited.

It is interesting to compare the normal ranges found by us in cows and ewes with the figures given by King & Armstrong [1934] for man and the dog. This is possible since the units were the same in both investigations. The normal range found by King & Armstrong for 24 normal human subjects between 19 and 42 years of age was 3.7-13.1 units, the majority falling between 5 and 10 units. For 21 dogs they found a wider range, two of them showing values of 27.4 and 32.1 units respectively, findings reminiscent of our results in ruminants.

Relation between serum phosphatase and age

Kay [1930, 1], Smith & Maizels [1932] and Stearns & Warweg [1933] reported that serum phosphatase was higher in children than in adults and similar age differences have been found in the rabbit and rat [Kay, 1930, 1] and dog [Bodansky, 1934]. The higher level in infancy may be associated with the more rapid skeletal development at this stage of life. Mulay & Hurwitz [1938] studied the plasma phosphatase in 300 human subjects ranging in age from the time of birth to 86 years and found comparatively high values between 1 and 15 years, a decrease between 15 and 25 years and thereafter a fairly constant level. Our data in general are in accord with the findings of these workers.

The data for Dairy Shorthorn cows were classified among the age groups, 2-4 years, 4-6 years and over 6 years. To these three groups were added a small group of heifers 9-24 months old, and three sets of results obtained on the same population of calves at three age periods. These data are given in Table 2.

Table 2. *Distribution of serum phosphatase in different age groups of Dairy Shorthorn cattle*

Serum phosphatase units	Calves 8 weeks	Calves 17 weeks	Calves 27 weeks	Heifers 9-24 months	Cows 2-4 years	Cows 4-6 years	Cows over 6 years	Total of calves	Total of heifers	Total of cows	Total of cows and heifers
0.0-4.9	—	—	—	—	1	3	1	—	—	5	5
5.0-9.9	—	—	1	—	15	12	6	1	—	33	33
10.0-14.9	2	3	14	8	8	3	3	19	8	14	22
15.0-19.9	8	14	8	4	2	3	3	30	4	8	12
20.0-24.9	6	8	1	2	1	4	2	15	2	7	9
25.0-29.9	8	2	—	3	—	—	2	10	3	2	5
30.0-39.9	3	3	—	—	—	1	1	6	—	2	2
40.0-49.9	4	—	—	1	2	—	1	4	1	3	4
50.0-99.9	1	—	—	—	1	—	6	1	—	7	7
100.0 and over	—	—	—	—	1	—	—	—	—	1	1
Total no.	32	30	24	18	31	26	25	86	18	82	100

Among the calves, data for which are recorded in the first three columns, there is at first a wide scatter of values from 10 to 50 units with a subsequent tendency towards a closer scatter and a downward trend of values. The individual records of 24 of the calves which were bled on all three occasions show that 22 of them yielded diminishing values at successive samplings, while the values for the other 2 were singularly steady throughout the period. The heifers form a small intermediate group and the three age groups of cows show a preponderance of even lower values up to 6 years old, after which there is again a number of higher values. The groups of animals studied here are too small to warrant much discussion, but the data do suggest that in cattle, after maturity, there is not likely to be any regular trend in the serum phosphatase level. It is admitted that the composite picture of the relation between age and serum phosphatase shown in Table 2 is far from complete, and the data do not permit

the drawing of hard and fast conclusions. The only way to obtain a satisfactory picture of the change of serum phosphatase with advancing age is to study a large number of animals from birth to old age, a long and difficult task in the case of cattle.

In addition to the data on cattle there are similar data for sheep and these are summarized in Tables 3, 4 and 5.

Table 3. *Serum phosphatase data in four Suffolk x Dorset, Horn lambs from birth to about 10 weeks old*

Lamb no.	Age in days							
	1	7	14	21	27	36	51	67
911	264	90	57	45	72	73	68	32
912	181	135	47	65	89	86	72	30
913	226	64	64	40	43	22	18	13
914	229	134	127	84	54	41	31	16
Average	225	106	74	58	65	55	48	23

Table 4. *Average values of serum phosphatase in 10 Cheviot lambs from 25 to 185 days old*

Average age in days	25	43	51	58	71	85	112	161	185
Range of phosphatase	64-165	60-127	41-150	41-115	32-77	27-73	11-27	9-34	5-33
Average phosphatase	105	84	92	83	50	40	20	20	13

Table 5. *Percentage distribution of serum phosphatase in 7 age groups of sheep from Newcastle 'pining' experiment*

Serum phosphatase units	5 months old 139 samples	9 months old 43 samples	13 months old 69 samples	17 months old 89 samples	3 years old 92 samples	3½ years old 87 samples	4 years old 93 samples
0.0- 4.9	—	—	—	—	1.0	—	2.1
5.0- 9.9	4.3	2.3	1.4	16.9	21.7	6.9	42.0
10.0- 14.9	8.6	23.2	23.2	24.7	23.9	41.4	26.9
15.0- 19.9	15.1	23.2	31.9	16.9	20.7	18.4	5.4
20.0- 24.9	18.7	16.3	8.7	15.7	6.5	15.0	8.6
25.0- 29.9	15.1	23.2	10.1	7.9	7.6	5.7	3.2
30.0- 39.9	21.6	7.0	18.9	11.2	7.6	5.7	6.4
40.0- 49.9	12.9	2.3	1.4	1.1	6.5	4.6	2.1
50.0- 99.9	1.4	2.3	2.9	3.4	3.3	1.1	2.1
100.0-199.9	2.2	—	1.4	—	1.0	1.1	1.1
200.0-349.9	—	—	—	1.1	—	—	—
Under 15 units	12.9	25.2	25.6	41.6	46.6	48.3	71.0
Under 40 units	83.4	95.4	94.3	94.4	89.2	93.2	94.7

Tables 3 and 4 show that at birth the lamb has a very high serum phosphatase which falls very rapidly in some cases and more slowly in others, the fall continuing up to about 6 months of age. There is a decided difference in actual level in the two groups at corresponding ages but the trend is much the same in each group. These groups are too small to enable us to say whether or not this difference is likely to be connected with the breed.

It will be noted (see Table 5) that while the percentage of values under 40 units is fairly constant after 9 months of age in the sheep, there is a more or less steady increase in the number of values under 15 units, which rises from 12.9% at 5 months to 71.0% at 4 years. This shows a progressive condensing

of the scatter of phosphatase values with advancing age, a finding not observed in the smaller and differing cattle populations studied. The general picture emerging from the data on sheep resembles in part the finding in cattle but is more clear cut, possibly because the life of the sheep is shorter than that of the cow and a larger proportion of the life-span has been studied. The sheep data can furthermore be considered as of more significance than the data on cows in that the same population of sheep was studied over the last three ranges of age, whereas the data in Table 2 were obtained on three different and smaller groups of cattle. The possible existence of a species difference in the relation between age and serum phosphatase, nevertheless, cannot be entirely ignored.

The serum phosphatase level of the individual

In view of the wide overall range of values found among cattle and sheep it was considered important to know also what variability occurred from time to time in the same individual. A certain number of cows, ewes and lambs have been bled on a number of occasions and the full data available for these animals are presented in Tables 6 and 7.

Table 6. *Serum phosphatase values of 11 dairy Shorthorn cows at successive samplings*

Name of cow	1934			1935					Range of values	Average	
	1. xii.	5. xii.	10. xii.	14. i.	23. i.	11. ii.	11. iii.	8. v.			18. xi.
Campion 2	23	—	24	21	—	15	17	24	35	15- 35	23
Carlet 3	—	—	58	—	—	57	56	53	114	53-114	68
Lily 3	—	11	11	10	—	11	17	11	12	10- 17	12
Flora 1	—	—	9	8	—	9	9	—	—	8- 9	9
Linda 5	—	—	61	—	—	47	58	68	—	47- 68	58
Patricia	8	—	11	—	—	10	10	13	—	8- 13	10
Peach 3	48	—	44	48	—	43	53	70	63	43- 70	53
Peach 5	—	17	17	—	29	28	27	21	44	17- 44	26
Pippin	—	—	22	26	23	18	27	27	—	18- 27	24
Red Rose	—	—	9	—	—	8	9	12	12	8- 12	10
Rosalie	—	—	42	44	—	40	49	35	67	35- 67	46

From the data in these tables it appears that the general level of serum phosphatase in the normal adult individual remains sensibly constant over periods of the order of at least 1 year in the case of cows and 18 months in the case of ewes and lambs. An animal showing a low serum phosphatase activity at the first sampling has usually continued to do so with moderate fluctuations around the mean level, whereas an animal showing a high initial value always showed a high value. It is further clear that those animals showing high values, e.g. Carlet 3, Linda 5, Peach 3 and Rosalie in Table 6 and Lambs 46, 71, 75, and 136 and Ewe 26 in Table 7, also showed considerably wider fluctuations in serum phosphatase than animals showing a generally low level. As far as we are able to tell, the level of serum phosphatase of the individual, whether high or low, does not seem to be associated with any clinical abnormality. The wide variation in general level of serum phosphatase among apparently normal cattle and sheep is, however, a matter of great interest, and the possibility that changes in serum phosphatase might be associated with physiological conditions such as pregnancy and lactation was examined.

Table 7. *Serum phosphatase values in 41 sheep at 7 different samplings showing individual variation*

No.	Nov. 1936	Dec. 1936	Jan. 1937	Feb. 1937	July 1937	Nov. 1937	Mar. 1938	Range	Average
1936 lambs									
2	7	14	9	11	12	13	10	7-14	11
6	13	19	13	15	19	18	8	8-19	15
12	40	35	30	23	30	33	17	17-40	30
15	20	36	25	33	31	30	26	20-36	29
39	20	16	16	12	16	12	11	11-20	15
40	10	15	12	13	18	10	11	10-18	13
44	8	17	10	10	15	7	8	7-17	11
46	102	129	205	156	52	131	89	52-205	124
67	27	22	8	9	23	15	8	8-27	16
71	46	120	38	55	38	72	44	38-120	59
75	122	146	57	119	—	19	—	19-146	93
77	13	17	13	9	15	11	9	9-17	12
99	47	38	20	25	29	28	23	23-47	30
101	26	19	14	13	29	14	11	11-29	18
102	27	21	13	17	12	15	9	9-27	16
108	35	30	14	16	21	17	8	8-35	20
131	39	26	21	23	26	25	15	15-39	25
134	50	19	13	9	19	16	11	9-50	19
136	142	—	—	—	172	316	—	142-316	—
139	36	48	24	35	14	30	37	14-48	32
142	68	46	15	28	—	40	25	15-68	36
1936 ewes									
19	17	18	14	10	14	9	8	8-18	13
22	7	10	8	8	9	9	9	7-10	8
26	110	166	62	135	51	113	114	51-166	107
32	41	49	31	25	45	29	37	25-49	34
49	9	11	7	8	11	6	6	6-11	8
52	29	15	15	14	37	21	16	14-37	21
60	20	14	9	7	10	9	8	7-20	11
62	52	34	21	22	43	25	15	15-52	30
84	11	12	5	9	10	10	6	5-12	9
86	17	9	14	15	—	3	9	3-17	11
91	26	27	13	16	19	16	9	9-27	18
96	42	25	19	17	25	22	21	17-42	24
113	17	22	7	7	10	14	8	7-22	12
120	44	26	23	28	36	33	22	22-44	30
122	16	17	8	13	14	11	7	7-17	12
124	43	17	13	24	23	22	15	13-43	23
149	9	12	6	8	11	11	7	6-12	9
152	9	8	5	5	9	6	6	5-9	7
154	15	18	5	7	17	12	11	5-18	12
156	17	20	10	7	19	7	9	7-20	13

Serum phosphatase and pregnancy in the dairy cow

Wilson & Hart [1932] and Auchinachie & Emslie [1933] claim that serum phosphatase is lower in pregnant than in non-pregnant cows, while the latter authors also found the same to hold for ewes. Vermehren [1939] stated that in women serum phosphatase is increased in the latter half of pregnancy, reaching a maximum just before parturition and falling sharply immediately *post partum*. He further states that the level during lactation is somewhat higher than the normal level.

Our available data for the dairy cow have been classified in Table 8 in two ways: (a) according as to whether or not the cow was pregnant when sampled, and (b) the values for pregnant cows according to stage of pregnancy.

Table 8. *Serum phosphatase of dairy cows in relation to pregnancy*

Serum phosphatase units	Pregnant		Non-pregnant		Nos. at various stages of pregnancy		
	No.	%	No.	%	0-95 days	96-190 days	191-285 days
0.0- 4.9	3	5.2	1	1.6	1	1	1
5.0- 9.9	10	17.5	27	43.5	2	6	2
10.0-14.9	15	26.3	11	17.7	9	5	2
15.0-19.9	5	8.8	8	12.9	2	1	2
20.0-24.9	3	5.2	4	6.4	2	1	—
25.0-29.9	4	7.0	3	4.8	2	1	1
30.0-39.9	1	1.8	1	1.6	1	—	—
40.0-49.9	9	15.8	1	1.6	1	6	2
50.0-99.9	6	10.5	5	8.0	3	1	1
100.0 and over	1	1.8	1	1.6	—	—	1
Totals	57		62		23	22	12
Under 15 units		49.0		62.8	12	12	5
Under 40 units		71.9		88.8	19	15	9

Comparing the serum phosphatase levels in pregnant and non-pregnant cows in Table 8, it is noted that there is a higher proportion of low values among the non-pregnant cows and that the scatter of values is wider among the pregnant cows, suggesting at first sight a tendency towards higher serum phosphatase values in pregnancy. Our data, therefore, are more in line with those of Vermehren [1939] on women than with those of Wilson & Hart [1932] and Auchinachie & Emslie [1933] on cattle and sheep. It must, however, be admitted that our data are not sufficiently extensive to preclude the possibility of a decrease in serum phosphatase in late pregnancy such as was suggested by the results of Auchinachie & Emslie [1933] on six cows sampled during the fifth and eighth months of pregnancy. The data given in the last three columns of Table 8 do not suggest the existence of any pronounced relation between stage of pregnancy and serum phosphatase level. This, taken in conjunction with the individual records of cows sampled when pregnant and non-pregnant, suggests that the preponderance of low values in non-pregnant cows may be due to chance, though the possibility remains that the physiological condition of pregnancy irrespective of the stage reached is associated with a tendency towards an increase in serum phosphatase.

Serum phosphatase of the dairy cow in relation to milking capacity

In searching for possible causes of the wide individual differences in serum phosphatase of dairy cows it was thought that strain on the Ca and P metabolism due to the large daily excretion of Ca in the milk of high-yielding cows might be reflected in an abnormally high serum phosphatase level [cf. Vermehren, 1939]. Accordingly, available data relating to milk yield and serum phosphatase are presented in Tables 9 and 10.

In Table 9 the cows are classified into two groups, according to whether the yield in the lactation previous to sampling was more or less than 7500 lb.

Though the average lactation yields of the two groups of cows differ widely, the distributions of serum phosphatase values are so similar as to suggest that there is no association between milk yield and serum phosphatase. This conclusion is confirmed by the more detailed data (see Table 10) on 11 Dairy Shorthorn cows of which 5 gave over 9000 lb. and 6 gave under 9000 lb. in the previous lactation.

Table 9. *Average values of serum phosphatase in dairy cows of two different grades of milking capacity*

Serum phosphatase units	Total milk yield in lactation previous to the samplings			
	Under 7500 lb.		Over 7500 lb.	
	No.	%	No.	%
0.0- 4.9	24	29.3	16	26.3
5.0- 9.9	29	35.4	21	34.4
10.0-14.9	9	11.1	8	13.2
15.0-19.9	6	7.3	4	6.5
20.0-24.9	5	6.1	3	4.9
25.0-29.9	1	1.2	2	3.3
30.0-39.9	2	2.4	1	1.6
40.0-49.9	3	3.6	1	1.6
50.0-99.9	3	3.6	5	8.2
Totals	82		61	
Highest yield in lb.	7463		16454	
Lowest yield in lb.	1142		7626	
Average yield in lb.	5830		9224	

Table 10. *Serum phosphatase of 11 Dairy Shorthorn cows of different grades of milking capacity*

Cows giving over 9000 lb. in last lactation				Cows giving under 9000 lb. in last lactation			
Name	Milk yield lb.	Range of serum phosphatase	Average serum phosphatase	Name	Milk yield lb.	Range of serum phosphatase	Average serum phosphatase
Carlet 3	13036	53-114	68	Pippin	8864	18-27	24
Peach 3	12358	43- 70	53	Red Rose	8638	8-12	10
Lily 3	9759	10- 17	12	Linda 5	8438	47-68	58
Peach 5	9593	17- 44	26	Patricia	8000	8-13	10
Rosalie	9321	35- 67	44	Campion 2	6868	15-35	23
				Flora 1	4043	8- 9	9
Average	10813		40.6		7142		22.3

Table 11. *Serum phosphatase data of 9 herds representing 6 breeds of cattle*

Serum phosphatase units	Dairy Shorthorn herds				Guernsey herds			Other dairy breed herds				Herefords
	S ₁	S ₂	S ₃	Total	G ₁	G ₂	Total	D	A	F	Total	
0.0- 4.9	—	2	8	10	4	3	7	—	16	26	42	38
5.0- 9.9	20	17	18	55	18	27	45	9	4	4	17	60
10.0-14.9	22	2	9	33	8	14	22	3	—	2	5	12
15.0-19.9	11	2	5	18	2	6	8	1	—	—	1	1
20.0-24.9	10	—	5	15	—	7	7	—	—	—	—	3
25.0-29.9	9	—	6	15	—	1	1	1	—	—	1	3
30.0-39.9	3	2	10	15	—	3	3	2	—	—	2	2
40.0-49.9	10	2	4	16	—	3	3	1	—	—	1	—
50.0-99.9	12	4	5	21	—	1	1	—	—	—	—	—
100.0 and over	2	—	1	3	—	—	—	—	—	—	—	—
Totals	99	31	71	201	32	65	97	17	20	32	69	119

Normal range of serum phosphatase in various breeds of cattle

Data are available (see Table 11) for cows of 6 breeds comprising 3 herds of Dairy Shorthorns (S₁, S₂ and S₃), 2 herds of Guernseys (G₁ and G₂), and 1 herd each of South Devons (D), Ayrshires (A), British Friesians (F) and Herefords (H).

From these data it appears that the serum phosphatase distribution for Dairy Shorthorns differs from that for cows of other breeds in that it is the only breed

exhibiting values over 100 units. Further, about 10% of values for Shorthorns lie between 50 and 100 units compared with only 1% in the case of Guernsey cows and none in the other breeds. This wide scatter of values in the Dairy Shorthorn was first observed in herd S₁, and herds S₂ and S₃ were then studied to find out if the peculiarity was a general characteristic of the breed or merely a peculiarity of the particular herd associated with conditions of management or breeding. Since, however, the distribution was similar in all 3 herds it seems fair to assume that it is a genuine breed characteristic.

Data for Ayrshire and British Friesian cattle are comparatively slender, but it is noteworthy that the values for these breeds are all under 15 units with the great majority of values under 5 units. Intermediate between these breeds and the Dairy Shorthorns come the Guernseys, South Devons and Herefords, the last-mentioned being a 'beef' breed. The herd studied by us was at grass all the year round and kept for calf rearing only. We do not propose to comment further on this breed difference since at present no explanation for it can be offered.

DISCUSSION

One feature of the data which have been summarized in the foregoing tables is of such interest as to merit further discussion. We refer to the surprisingly scattered distribution of the serum phosphatase values in ewes and in cows of the Dairy Shorthorn, and, to a less extent, the Guernsey and South Devon breeds. It is difficult to give any adequate explanation of this phenomenon though one or two possibilities will be considered.

There is a certain amount of evidence which suggests that individual differences in the level of thyroid activity might be responsible for the wide variation in serum phosphatase levels of apparently normal individuals. Kay [1930, 2] found that the serum phosphatase was raised above normal in cases of exophthalmic goitre in man. Scoz & Marangoni [1934] observed a transient rise in plasma phosphatase in one dog after thyroxine treatment. Folley & White [1936] noted an increase in serum phosphatase in lactating cows during thyroxine treatment, but concluded that the changes were not large enough to account for the individual variations in serum phosphatase which had been observed at Shinfield among Dairy Shorthorn cows.

A second possibility is opened up by the observation of Folley [1936] that injection of large doses of oestrogenic hormones caused a temporary rise of the order of 50% in serum phosphatase accompanied by a fall in serum Ca in lactating cows. These changes were interpreted as indicating that oestrogens are capable of exerting an influence on the skeletal system, a contention which has since been repeatedly confirmed [cf. Gardner & Pfeiffer, 1938]. Despite the fact that the doses of oestrogen used in Folley's [1936] experiments were unphysiological and the changes in serum phosphatase not large, the remote possibility remains that individual differences in serum phosphatase might be due in some cases to differences in ovarian activity.

Lastly we would discuss the possibility that the high phosphatase activity of some sera is due to the presence of a phosphatase activator. This possibility was considered by one of us (S. J. F.) but discarded on the basis of evidence acquired in 1935. It was claimed by Thannhauser *et al.* [1937, 1] that ascorbic acid was such an activator, but King & Delory [1938] failed to confirm this and the claim was withdrawn [Thannhauser *et al.* 1938, 1]. In view of the more recent paper of Thannhauser *et al.* [1938, 2] in which the authors claim to have obtained evidence of the presence in sera showing high phosphatase

activity of a substance which, when added to normal sera, causes an increased hydrolysis of phosphoric ester, further discussion of this question seems called for.

In investigating this question at Shinfield in 1935, a serum of relatively high phosphatase activity and two of normal or low activity were used. King & Armstrong [1934] have shown that phosphatase estimations carried out by their method on undiluted sera showing an activity of 60 or more of their units per 100 ml., tend to be low owing to inhibition of the activity of the enzyme by the inorganic phosphate produced by the hydrolysis. Accordingly the high phosphatase serum was diluted with an equal volume of normal saline, this being the normal procedure specified by King & Armstrong for sera showing high phosphatase activity.

The phosphatase activities of the three sera, as well as of mixtures of equal parts of diluted high phosphatase serum with each of the other two, were determined. It was considered that if the highly active serum contained an activator, the activity of a mixture of this with a serum of normal activity would be greater than the arithmetic mean of the activities of the two components. This was essentially the procedure later adopted by Thannhauser *et al.* [1938, 2] to investigate this question. The actual figures obtained are given in Table 12, from which it will be seen that the differences between the theoretical and actual values for the phosphatase activities of the mixtures are well within the limits of experimental error. It would therefore appear that the relatively high phosphatase activity of one of these three sera cannot be ascribed to the presence of an enzyme activator.

Table 12. *Effect of mixing sera of different phosphatase values*

Actual values: serum 1 (diluted 1 : 1), 24.7 units per 100 ml.; serum 2, 9.7 units per 100 ml.; serum 3, 8.9 units per 100 ml.

	Theoretical value	Value found
By mixing equal volumes of 1 and 2	17.2	17.7
By mixing equal volumes of 1 and 3	16.9	17.3
By mixing equal volumes of 2 and 3.	9.3	9.2

Thannhauser *et al.* [1938, 2] used a method for the determination of phosphatase activity [see Thannhauser *et al.* 1937, 1] based on the method of Bodansky [1933] but the details of technique are not given fully. In their paper they report that the amount of phosphoric ester hydrolysed by mixtures of equal volumes of normal sera agreed closely with expectation but that when mixtures of normal sera and highly active sera were used the phosphate produced exceeded the expected amount. This was taken as evidence of the presence of a phosphatase activator in the pathological sera. In the absence of full experimental details it is hard to make critical comment on their data, but the fact that the more the highly active serum was diluted with normal serum the more the phospholytic activity of the mixture exceeded the theoretical value, suggests that the true activities of the highly active sera might have been underestimated, on account of the slowing up of the rate of hydrolysis due to the inhibiting action of the end-products, a point guarded against in the technique used by us. Against this possible explanation must be set the statement that when four times as much serum was used, the quantity of ester hydrolysed was proportionately greater, which, if other conditions such as concentration of substrate, total volume of liquid etc. were the same in both cases, would preclude the likelihood of their original determinations on highly active sera having been in error due

to the inhibitory action on the enzyme of the inorganic phosphate liberated. A further point should be borne in mind. The highly active sera of Thannhauser and his colleagues were obtained from cases of Paget's disease in man and from dogs which had undergone ligation of the common bile duct, whereas the results in Table 12 were obtained with sera from clinically normal cows. It is possible that in Paget's disease, or after ligation of the common bile duct, the blood may contain a phosphatase activator which is absent from blood showing high phosphatase activity due to other causes, or that such an activator may be present in highly active sera from man and carnivores but not from ruminants. In support of this possibility it may be mentioned that there is some evidence of species differences as regards changes in serum phosphatase. Thus Thannhauser *et al.* [1937, 2] claim that ligation of the common bile duct causes an increase in serum phosphatase in dogs but not in cats, while rickets is accompanied by an increase in serum phosphatase in man but not in the rat (Prof. H. D. Kay, personal communication). Whatever may be the true explanation of the results of Thannhauser and his colleagues, the postulation of the presence of an activator in highly active sera does not seem to be a likely explanation of the wide range of values encountered among apparently normal cows of the Dairy Shorthorn breed and among ewes.

SUMMARY

1. In apparently normal cows and ewes the variations in serum phosphatase, 0.3-114.3 and 3.0-166.1 units per 100 ml. respectively, are so wide as to lead to the conclusion that its determination can be of little diagnostic significance.

2. Cows of the Dairy Shorthorn breed show a wider range of values and a greater proportion of high values than cows of four other dairy breeds and one beef breed. No explanation of this breed difference can be offered.

3. Though the serum phosphatase may differ widely from animal to animal the general level of the individual remains sensibly constant over long periods of time.

4. In both cattle and sheep serum phosphatase activity progressively decreases with advancing age until maturity is reached. Thereafter cattle show little alteration but there is some evidence in sheep of a further slight decrease with advancing age.

5. There is some evidence that pregnancy *per se* is accompanied by a slightly raised serum phosphatase in dairy cows, though the level of phosphatase does not appear to be correlated with the stage of pregnancy.

6. No evidence was found of correlation between milking capacity and serum phosphatase activity in dairy cows.

7. The high phosphatase activity of the sera of some clinically healthy cows does not appear to be due to an enzyme activator in these sera.

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