

L. THE EFFECT OF DIET ON THE PLASMA-PHOSPHATASE OF SHEEP.

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RECENT work on the rôle of phosphatase has made it clear that the enzyme is closely concerned in the metabolism of calcium and phosphorus and in bone formation. Thus, it has been shown that an increased plasma-phosphatase content accompanies certain bone diseases in human beings [Kay, 1930], that a rise in value also occurs during the healing of fractures [McKeown and Ostergren, 1931], and that, during the healing of rickets in children, the phosphatase values are still abnormally high even after the serum-Ca and blood-inorganic P have returned to normal [Smith and Maizels, 1932]. Bodansky and Jaffe [1931] observed that certain dietary deficiencies were accompanied by changes in the serum-phosphatase of rats.

In view of the marked changes which have been found in the serum-Ca levels of sheep suffering from experimentally produced "bent-leg" [Auchinachie and Fraser, 1932], it was considered desirable to determine whether the plasma-phosphatase of sheep fed on the same or similar diets would be affected in any way.

EXPERIMENTAL.

Blood samples were drawn at monthly intervals, and the plasma-phosphatase, serum-Ca, and blood-inorganic P values were determined. Plasma-phosphatase was estimated by the method of Kay [1930], serum-Ca by Tisdall's modification of the Kramer-Tisdall technique, and inorganic P by Havard and Reay's modification of Briggs's adaptation of the Bell-Doisy method. The sheep were also weighed at monthly intervals.

Exp. 1. It was obviously essential in an experiment of this nature and duration to consider the possibility of seasonal changes. During the course of the experiment the blood of normal sheep fed on adequate diets was analysed. The average results are given in Table I. It was not possible to get samples from

Table I. *Blood analyses of normal sheep at different months.*

Month	Source	No of animals	Units phosphatase Av.	Range	mg./100 cc.	
					Serum-Ca	Blood-inorganic P
March	Institute	4	0.264	(0.226-0.350)	10.58	6.71
June	Slaughter-house	11	0.335	(0.081-0.388)	9.48	4.22
October	Institute	34	0.255	(0.169-0.347)	10.42	5.79
November	Institute	4	0.283	(0.276-0.291)	10.20	5.11
December	Institute	37	0.226	(0.127-0.323)	11.12	5.22

normal sheep during the summer months, but blood from slaughter-house sheep was analysed instead. The rather high plasma-phosphatase values and low serum-Ca and blood-inorganic P, however, would appear to indicate that the slaughter-house animals were in a rather poor nutritional state.

It can be seen from these results that there is no evidence of a definite seasonal change in plasma-phosphatase. The differences noted in the subsequent experiments can, therefore, be attributed only to dietary factors and not to seasonal changes. Furthermore, as the sheep in these experiments were housed indoors, they were all the less likely to be affected by climatic differences.

Exp. 2. Four half-bred (Border Leicester \times Cheviot) wether hoggs¹, strictly comparable as regards weight, age (7 months) and general health, were housed indoors and divided into two groups. The bedding consisted of moss litter, and dietary control was complete.

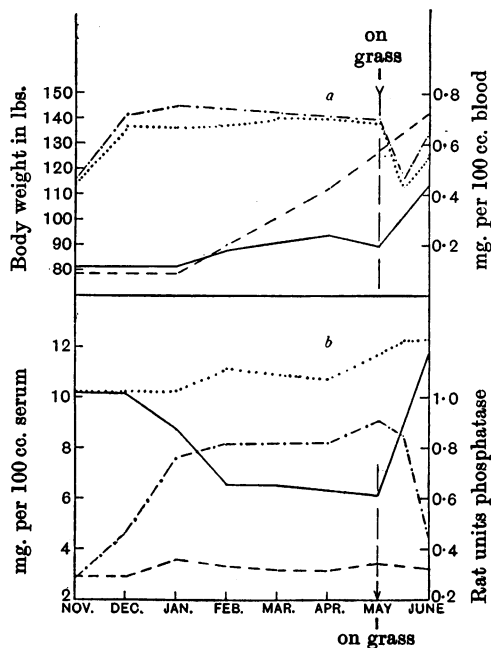


Fig. 1.

- (a) Body weight in lbs. (complete ration) — — — —
 Body weight in lbs. (basal ration) — — — —
 Inorganic P (complete ration)
 Inorganic P (basal ration) — — — —
- (b) Serum-Ca (complete ration)
 Serum-Ca (basal ration) — — — —
 Phosphatase (complete ration) — — — —
 Phosphatase (basal ration) — — — —

Group I was given the basal ration described by Auchinachie and Fraser [1932]. It was deficient in calcium but high in phosphorus when compared with good cultivated pasture, the ratio $\text{CaO} : \text{P}_2\text{O}_5$ being 1 : 4.17, whereas good pasture usually has a ratio of the order 1 : 0.77 (on a dry matter basis).

¹ Immature castrated males.

Group II received the basal ration supplemented with

(a) ground limestone (the quantity given, 0.5 oz. per sheep per day, raised the lime intake from 2.7 to 9.8 g. per day):

(b) cattle cod-liver oil, 2 cc. per sheep per day.

As will be seen from Fig. 1, definite changes in the three blood constituents studied were established after 2 months, and after 6 months they were much more marked. As the animals in Group I were then in very poor condition, the experimental feeding was discontinued, and the sheep in both groups were turned out to pasture, two further blood analyses being carried out at intervals of 14 days.

The average results of Exp. 2 are shown in Fig. 1, from which it can be seen that, 1 month after the experiment had commenced, the plasma-phosphatase content of the sheep in Group I showed a marked increase in value, unaccompanied by any appreciable change either in Ca, P or body weight. Indeed, it was only after 2 months that the serum-Ca had fallen appreciably relative to the original values, and it was a little after this that clinical evidence of malnutrition first became evident. The average plasma-phosphatase value in this group had, by this time, reached a level of 0.761 unit, nearly 3 times the original (0.283 unit), and it continued to rise slowly until it reached a value of 0.903 unit in May, when the artificial diet was discontinued. Except for a slight retardation, the Ca curve of Group II on the adequate diet is almost a mirror image of the phosphatase curve. The same negative correlation is seen to have occurred after the sheep were turned out to pasture, but the serum-Ca returned to normal levels, while the plasma-phosphatase still remained at a level higher than its initial value. In Group II neither of these constituents of the blood underwent any appreciable change except for a very slight fall in plasma-phosphatase and rise in serum-Ca after the sheep had been on pasture. The inorganic P showed no changes corresponding to changes in diet, for there was a rise in both groups during the experimental feeding. This increase was doubtless due to the high phosphorus content of the ration and therefore agrees with the result of the previous experiment (Auchinachie and Fraser). The weight curves bring out clearly the inadequacy of the basal ration (Group I) as compared with the supplemented ration, which appears to possess as good growth-promoting properties as good cultivated pasture.

Exp. 3. This experiment was begun for another purpose, and plasma-phosphatase determinations were begun only when it became evident from Exp. 2 that alterations in plasma-phosphatase content were likely to be brought about by deficient diets. Only two sets of values are, therefore, available, those for March and April.

Thirty-six healthy black-faced sheep, 7 months old, were divided into 6 equal groups which were placed on the diets outlined in Table II. Pre-experimental phosphatase values were not estimated, but those obtained as a standard of comparison in Exp. 2 can safely be employed as they refer to the same time of the year as that in which Exp. 3 was begun (*i.e.* November).

It will be seen that the Ca and phosphatase values obtained for the basal and basal + CaCO₃ groups agree very closely with those obtained for the basal group in Exp. 2, but the addition of CaCO₃ alone to the basal ration apparently checked to some extent the fall in serum-Ca and rise in plasma-phosphatase. The addition of 2.5 cc. of cod-liver oil (Group 3) to the basal ration was, however, of much greater benefit in this respect, the level of plasma-phosphatase being normal, although the serum-Ca values were slightly lower than those of sheep fed both CaCO₃ and cod-liver oil. It would appear that 2.5 cc. of cod-liver oil

Table II.

Group	Diet	March		mg./100 cc.		April		mg./100 cc.	
		Units of phosphatase	Range	Serum-Ca	Blood-in-organic P	Units of phosphatase	Range	Serum-Ca	Blood-in-organic P
1	Basal	0.699	(0.535-0.776)	6.29	7.50	0.727	(0.615-0.863)	6.36	7.59
2	Basal + CaCO ₃ (2 %)	0.669	(0.543-0.814)	7.75	8.93	0.653	(0.564-0.777)	7.31	7.12
3	Basal + C.L.O. (2.5 cc. per sheep per day)	0.293	(0.182-0.385)	10.00	8.17	0.232	(0.189-0.315)	10.44	7.03
4	Basal + CaCO ₃ + C.L.O. (1 cc. per sheep per day)	0.360	(0.274-0.436)	11.24	7.97	0.390	(0.192-0.536)	11.20	7.74
5	Basal + CaCO ₃ + C.L.O. (2.5 cc. per sheep per day)	0.298	(0.216-0.389)	10.96	7.99	0.321	(0.272-0.388)	11.60	7.43
6	Basal + CaCO ₃ + C.L.O. (5 cc. per sheep per day)	0.360	(0.243-0.424)	10.38	8.13	0.322	(0.262-0.461)	11.14	7.50

per sheep per day are more conducive towards a normal phosphatase value than either 1 cc. or 5 cc. Again, as in Exp. 2, the inorganic P values are higher than those of sheep fed on good cultivated pasture (unpublished data).

Exp. 4. Effect of pregnancy on plasma-phosphatase. In the course of our survey of the plasma-phosphatase content of the blood of various types of animals, it was found that the value in pregnant cows was low. This led to a more thorough study of the influence of pregnancy. In Table III are given the

Table III.

Class of animal	Number	Units of phosphatase
Sheep (pregnant)	56	0.066 (0.022-0.099)
Sheep (non-pregnant)	37	0.226 (0.127-0.323)
Cows (5 months pregnant)	6	0.130 (0.090-0.158)
Cows (8½ months pregnant)	6	0.069 (0.051-0.088)
Cows (non-pregnant)	11	0.146 (0.109-0.207)

values for cows and sheep during the later stages of pregnancy, together with those of non-pregnant animals. It would appear from these results that the plasma-phosphatase value is lower during pregnancy. This is not apparent in the data recorded by Kay [1930] for human beings but is in general a confirmation of the findings of Wilson and Hart [1932] for dairy cows.

DISCUSSION.

It is clear from these experiments that disordered metabolism of Ca and P is associated with a marked rise in plasma-phosphatase. Thus, in Exp. 2, the value for sheep on the basal ration had risen from the pre-experimental level of 0.283 to 0.465 unit a month after the animals had been placed on a diet low in Ca and rich in P. This value went on increasing, at first rapidly and then more slowly, until a level of 0.903 unit was reached, when the deficient ration was withdrawn. It is important to note that the increase in plasma-phosphatase, following the inadequate diet, occurred appreciably earlier than the fall in serum-Ca, and, what is still more important, before any other sign of disordered metabolism had appeared. It would seem, therefore, that plasma-phosphatase estimations are of greater diagnostic value than the serum-Ca level or clinical observation in regard to the state of the Ca and P metabolism. This conception is supported by the fact that the plasma-phosphatase of the sheep on the deficient

diet fell less rapidly than the serum-Ca rose towards their respective normal levels when the sheep were put on pasture. This finding agrees with that of Smith and Maizels [1932] cited above.

Knowledge of plasma-phosphatase is not yet sufficiently far advanced to enable one to indicate all the factors concerned in maintaining it at normal levels. It is, however, obvious that something in cod-liver oil plays a large part in this regard; for, as Exp. 3 shows, the plasma-phosphatase level remained practically normal in those animals which received 2.5 cc. daily. It would also appear that an optimum amount of the oil is necessary, because the phosphatase level was nearer normal for animals receiving 2.5 cc. of cod-liver oil than for those which received either 1 cc. or 5 cc. daily. While it is probable that vitamin D is the chief beneficial factor in cod-liver oil, there seems some discrepancy between the effect on the metabolism of Ca and P caused by vitamin D supplied by cod-liver oil and by irradiated ergosterol preparations (unpublished data). Experiments to clear up this point are now in progress. The calcium content of the diet has also some effect on the mineral metabolism of the animal as judged by plasma-phosphatase, but to a much smaller extent than cod-liver oil.

The data available do not enable one to assess the significance of the low plasma-phosphatase in pregnancy in ruminants. It is probable that simultaneous estimations of foetal and maternal blood would throw some light on the matter.

SUMMARY.

1. The plasma-phosphatase of the blood of sheep fed on a ration low in Ca and high in P increased progressively to from 3 to 4 times its normal value, while the serum-Ca fell.
2. Plasma-phosphatase determinations give a much earlier indication of disordered Ca and P metabolism than either serum-Ca, blood-inorganic P, general state of health or body weight.
3. Plasma-phosphatase appears to be lower in pregnant than in non-pregnant ruminants.

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