Seasonal changes in the plasma retinol-binding holoprotein concentration of sheep

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1. Seasonal variations in plasma retinol-binding holoprotein concentration have been found in two groups of entire ewes and in a control group of wethers (castrated rams).
2. In each group retinol-binding holoprotein level was minimal in summer and increased to peak values in September just before the autumn breeding season.

Following the isolation and characterization of pure retinol-binding protein (RBP) from plasma (Kanai, Raz & Goodman, 1968; Kirby, White & Glover, 1971; Peterson, 1971) it has become possible to analyse the content of this physiologically-active transport form of retinol with much greater precision, either by the fluorimetric method (Glover, Moxley, Muhilal & Weston, 1974) or by radioimmunoassay (Smith & Goodman, 1971) or by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) than was possible by the older colour tests for retinol. The RBP with retinol attached is referred to as the holoprotein (holoRBP).

The concentration of RBP in the plasma of experimental rats under different nutritional conditions can vary quickly with a half-life of only 7 h (Muto, Smith, Milch & Goodman, 1972; Peterson, Rask, Östberg, Andersson, Kamwendo & Pertoft, 1973; Muhilal & Glover, 1974). Since retinol is essential for reproduction (Thompson, Howell & Pitt, 1964) it was considered of interest to assay plasma holoRBP in a seasonal breeding animal over the annual cycle to study the possibility that hormonal changes may influence its concentration. Sheep holoRBP has been purified in our laboratory and its distribution in some tissues determined (Glover, Jay & White, 1974) and changes found in the plasma holoRBP level of sheep over an annual cycle are reported here.

EXPERIMENTAL

Animals. For the preliminary study samples were obtained in the summer months of 1972 and 1973 from a group of seven ewes (Derby Gritstone) (group A) maintained on upland pastures at the Great Houses Experimental Farm, Ministry of Agriculture, Food and Fisheries, Helmshore, Rossendale, Lancs. A group of four Clun Forest ewes that lambed in March–April 1975 (group B) and a group of four wethers (castrated rams) (group C) maintained at the University of Liverpool Veterinary Field Station, Leahurst, Wirral, Merseyside, were then selected for study over one full year from January 1975. The wethers were used as controls with a view to determine

Table 1. *Changes in retinol-binding holoprotein concentration (μg/ml) during the summer months of 1972 and 1973 in a group of seven Derby Gritstone ewes maintained on upland pasture*

(Mean values with their standard errors, determined fluorimetrically (for details, see below))

	1972		1973	
	Mean	SE	Mean	SE
13 April	—		47·3	2·1
26 May	54·5	1·6**	—	
7 June	—		45·2	1·3
23 June	39·8	1·3	—	
19 July	38·3	1·8	—	
	(34·8	2·8)†		
20 July	—		53·6	3·1*
10 September	—		54·2	2·8**
14 September	47·6	2·2**	—	
	(52·5	2·2)†		
5 October	43·2	1·7*	—	
	(42·8	3·0)†		

Statistical significance of difference between mean value and minimum mean value obtained for the same year: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Values for total retinol determined by the trifluoracetic acid colour test (for details, see below) and expressed in equivalent amounts of retinol-binding holoprotein.

whether any changes found in ewes could be attributed to the action of sex hormones as the gonads developed.

Blood samples (5 ml) were taken from the jugular vein by syringe needle (gauge 16) directly into centrifuge tubes pretreated with heparin. The plasma was separated and maintained at − 20° during transfer to the Liverpool laboratory for analysis the next day.

Methods. The plasma was analysed in dupicate for holoRBP by the fluorimetric procedure previously described (Glover, Moxley *et al.* 1974). A number of the preliminary samples from group A were also analysed for retinol using the trifluoracetic acid colour test described by Neeld & Pearson (1963). This provided an additional check that most of the retinol present in the plasma was in fact attached to its specific carrier-protein.

RESULTS

The results of the preliminary study with group A ewes during the summer months of 1972 and 1973 are given in Table 1. Minimum values for plasma holoRBP were obtained in midsummer (July 1972 < 40 μg/ml, June 1973 45 μg/ml), increasing again in September to approximately 50 μg/ml. Although the differences between the midsummer and September concentrations were only approximately 20% they were highly significant ($P < 0.01$; t test). Values for total retinol concentration for three of the samples obtained in 1972, expressed in terms of holoRBP, confirmed that most of the vitamin is transported by its specific carrier-protein. The results of the detailed study with group B ewes and group C wethers are shown in Fig. 1. The concentration of holoRBP in the period January–March varied between 20 and 40 μg/ml plasma for both male and female animals but decreased towards the lower end of the range by midsummer, minimum values (20–25 μg/ml plasma) being obtained in July and

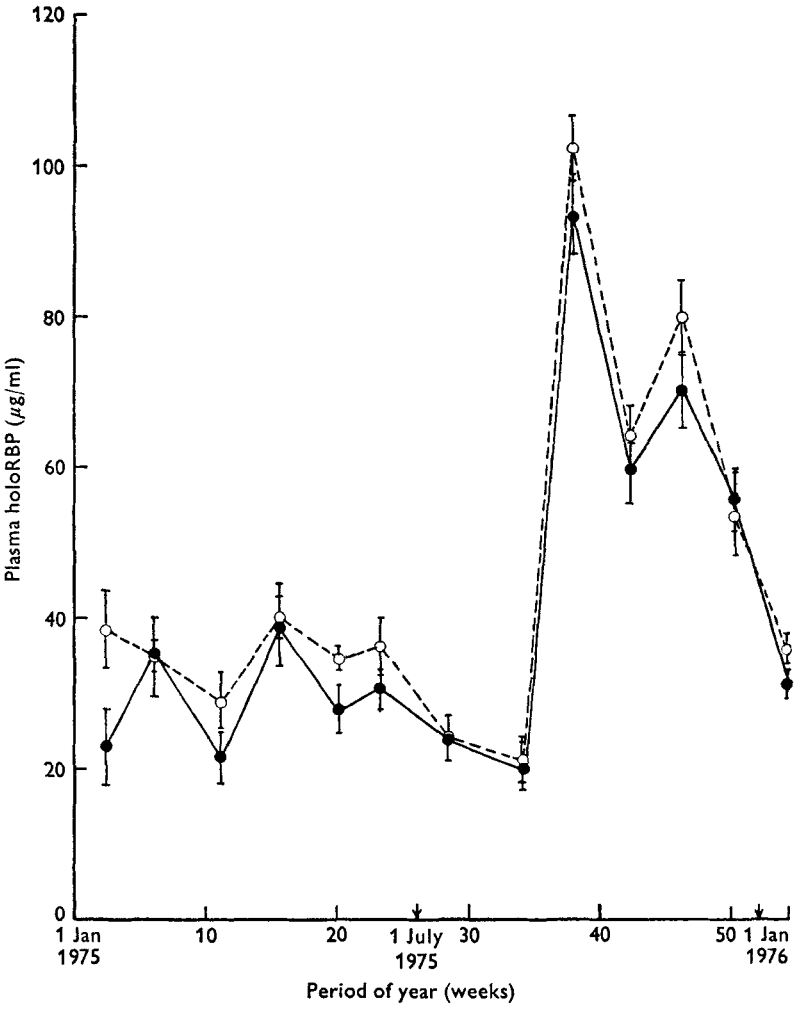

Fig. 1. Seasonal changes in plasma retinol-binding holoprotein (holoRBP) concentration in a group of four Clun Forest ewes which lambed in March–April 1975 (●—●), and a control group of four wethers (○– – –○) maintained on pasture. Samples were taken approximately mid-monthly and the holoRBP concentration was estimated fluorimetrically (for details, see p. 138). Mean values with their standard errors indicated by the vertical bars.

August. Immediately thereafter, however, the concentration of holoRBP in both sexes increased steeply to peak values (90–100 μg/ml plasma) in early September about threefold higher than the mean value for midsummer, thus confirming the increase found for the same period in previous years for group A ewes of a different breed. The concentration of holoRBP remained high throughout the autumn and then decreased in December to values similar to the January–February levels, thus completing the annual cycle. More detailed sampling was not done in March–April to avoid excessive interference with the ewes close to the termination of their pregnancy, although it has long been established in several species that retinol concentration (and

presumably RBP) decreases towards the end of pregnancy but recovers after parturition (reviewed by Moore, 1957).

The differences between the midsummer values and those for autumn and winter were again highly significant ($P < 0{\cdot}001$). The values for males in general tended to be slightly higher than the corresponding ones for females throughout the annual cycle. This sex difference has been noted previously in mean values for retinol in man (Kimble, 1939) and the rat (Moore, Sharman & Ward, 1951) and for RBP in man (Smith, Raz & Goodman, 1970).

The differences between the average level of holoRBP in group A (Derby Gritstone) ewes (35–45 μg/ml plasma) during the spring and summer of 1973 and 1974 and that (20–25 μg/ml plasma) for group B (Clun Forest) ewes in 1975 were probably the result of breed differences and of environmental conditions.

DISCUSSION

The results of these studies indicated that plasma concentrations of holoRBP in sheep did not remain at a steady level throughout the year, but after passing through minimal values in summer underwent a marked increase in September. It is perhaps surprising that the holoRBP level was minimal during midsummer when the liver reserves of the vitamin are substantial after the sheep have been grazing on the spring and early pastures rich in β-carotene. Furthermore, the finding that the major fluctuation in holoRBP concentration occurred synchronously in both ewes and wethers is equally striking and indicated that this change was not mediated by gonadal hormones. The 'September' surge in holoRBP concentration appeared to take place just before the development of the gonads for the autumn breeding season, the timing of which is governed by the shortening of daylight hours (Marshall, 1937; Yeates, 1949). The concentration of holoRBP although variable remained high during the autumn and early winter period when this species is sexually active, but then decreased to lower levels in late winter and spring, when gonads regress again.

In view of these findings two important factors in retinol metabolism must be considered. The first is related to the control of the plasma level and the second to the true function of holoRBP. The 'September' increase in plasma level is probably the result of increased synthesis of the carrier-protein by the liver rather than by a decrease in its rate of clearance and metabolism by the kidney, since there was a large rapid increase in the concentration of holoRBP particularly in group C. This finding also suggested hormonal control which probably involves part of the pineal–hypothalamus–pituitary system known to mediate in gonadal development (Wurtman, Axelrod, & Kelly 1968). In parallel studies comparable changes in the level of plasma holoRBP have also been found in Japanese quail before their breeding season in the spring (J. Glover & S. Large, unpublished results). There are also certain similarities in the seasonal change in holoRBP in sheep with the increase in holoRBP which was found in the polyoestrus rat with the onset of puberty (Peterson, Nilsson, Östberg, Rask & Vahlquist, 1974). All these phenomena are now being studied more closely and will form the subject of separate reports.

Regarding the function of holoRBP, the sudden increase in level just when gonadal tissues begin to develop is consistent with the established finding in this Department that retinol as opposed to retinoic acid is necessary for reproduction (Thompson *et al.* 1964). It would appear that holoRBP has a special function in controlling the supply of the vitamin to these tissues when it is particularly needed for their rapid growth and development.

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REFERENCES

Glover, J., Jay, C. & White, G. H. (1974). *Vitams. Horm.* **32**, 215.
Glover, J., Moxley, L., Muhilal, H. & Weston, H. (1974). *Clinica chim. Acta* **50**, 371.
Kanai, M., Raz, A. & Goodman, DeW. S. (1968). *J. clin. Invest.* **47**, 2025.
Kimble, M. S. (1939). *J. Lab. clin. Med.* **24**, 1055.
Kirby, W., White, G. H. & Glover, J. (1971). *Biochem. J.* **123**, 31P.
Mancini, G., Carbonara, A. O. & Heremans, J. F. (1965). *Immunochemistry* **2**, 225.
Marshall, F. H. A. (1937). *Proc. R. Soc.* B **122**, 413.
Moore, T. (1957). *Vitamin A.* Amsterdam: Elsevier Publishing Co.
Moore, T., Sharman, I. M. & Ward, R. J. (1951). *Biochem. J.* **49**, xxxix.
Muhilal, H. & Glover, J. (1974). *Br. J. Nutr.* **32**, 549.
Muto, Y., Smith, J. E., Milch, P. O. & Goodman, DeW. S. (1972). *J. biol. Chem.* **247**, 2542.
Neeld, J. R. & Pearson, W. N. (1963). *J. Nutr.* **79**, 454.
Peterson, P. A. (1971). *J. biol. Chem.* **246**, 34.
Peterson, P. A., Nilsson, S. F., Östberg, F., Rask, L. & Valquist, A. (1974). *Vitams. Horm.* **32**, 181.
Peterson, P. A., Rask, L., Östberg, L., Andersson, L., Kamwendo, F. & Pertoft, H. (1973). *J. biol. Chem.* **248**, 4009.
Smith, F. R. & Goodman, DeW. S. (1971). *J. clin. Invest.* **50**, 2426.
Smith, F. R., Raz, A. & Goodman, DeW. S. (1970). *J. clin. Invest.* **49**, 1754.
Thompson, J. N., Howell, J. McC. & Pitt, G. A. J. (1964). *Proc. R. Soc.* B **159**, 510.
Wurtman, R. J., Axelrod, J. & Kelly, D. E. (1968). *The Pineal*, p. 199. New York: Academic Press.
Yeates, N. J. M. (1949). *J. agric. Sci., Camb.* **39**, 1.

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