Plasma Carotenoids and Fat Mobilization in Stall-fed Cattle

In the course of work on the metabolism of starving sheep it was found that there was an association between increased depot fat mobilization, as indicated by the plasma content of non-esterified fatty acid, and the appearance of a yellow pigmentation in the plasma[1]. This pigment was shown to be xanthophyll[2], and a statistically highly significant and reproducible relationship was demonstrated between plasma non-esterified fatty acid level and xanthophyll content[3]. The possibility was considered that xanthophyll is passively mobilized from adipose tissue during fat mobilization and it therefore appeared that a similar relationship might obtain in the cow where carotenoids are usually present in the plasma.

Although it would be difficult to investigate this possible relationship in the normal grazing cow where the plasma carotenoid content is already high, it was considered worthwhile to examine suitable plasma samples taken from stall-fed cattle which show much lower levels of plasma carotenoids. Also, since increases in plasma non-esterified fatty acid content are usually observed in the cow during late pregnancy and early lactation[4,5], it was anticipated that large fluctuations in plasma non-esterified fatty acid level would be observed immediately before and after calving. At this stage, therefore, it would be opportune to examine the carotenoid–non-esterified fatty acid relationship.

Sixteen pregnant Ayrshire cows on a daily ration of hay (10 lb.), sugar beet pulp (7 lb.) and concentrates (6 lb.) were blood-sampled at frequent intervals for another purpose involving the measurement of plasma non-esterified fatty acid concentration, but for this investigation only three samples from each cow were considered: (1) taken 3 weeks before the earliest calving date; (2) taken immediately before calving (14 cows only); (3) taken 1–6 days after calving at a time when the highest non-esterified fatty acid level was measured. Plasma non-esterified fatty acid concentration was estimated in each sample using a modification of the method of Dole[6], previously described[7]. The heptane-isopropanol extract of plasma which was obtained for this purpose contained non-esterified fatty acid and also carotenoid pigments. The absorbance of these extracts at about 450 mμ has been used to give an approximate measure of plasma xanthophyll content in sheep[3], and in this experiment, after extractions had been made in semi-darkness, absorbance values were used to determine changes in plasma carotenoid content.

The measurements were quite reproducible and for a bulked plasma sample obtained from normally grazing cows the mean absorbance at 450 mμ in a 40-mm cell for five replicate extractions was 0·477 ± 0·001 (*S.E.*). However, the first plasma samples obtained from the 16 experimental cows 3 or 4 weeks before calving, with a mean non-esterified fatty acid content of 222·5 ± 18·2 μequiv./l. yielded extracts having absorbance values at λ_{max} (446–450 mμ) of only 0·162 ± 0·008. This indicated that the plasma of these stall-fed cattle contained approximately one-third the normal level of carotenoids.

The second and third samples showed high mean non-esterified fatty acid concentrations, 730·7 ± 90·7 and 1,048·8 ± 79·5 μequiv./l., respectively, while individual values ranged from 260 to 1,880 μequiv./l. As shown in Fig. 1, there was a statistically significant correlation between plasma non-esterified fatty acid content and absorbance at λ_{max} for 30 individual plasma extracts ($r = +0{\cdot}743$; $P < 0{\cdot}001$). Extracts from the second and third samples were combined, evaporated to dryness, saponified in 0·5 N alcoholic potassium hydroxide and the extracted pigment subjected to thin-layer chromatographic analysis. Using either development on silica gel with chloroform : methanol (95 : 5 v/v)[3] or on liquid paraffin impregnated silica gel with methanol : acetone (8 : 2 v/v)[8], the pigment behaved chromatographically as a mixture of β- and γ-carotenes contaminated with traces of xanthophyll.

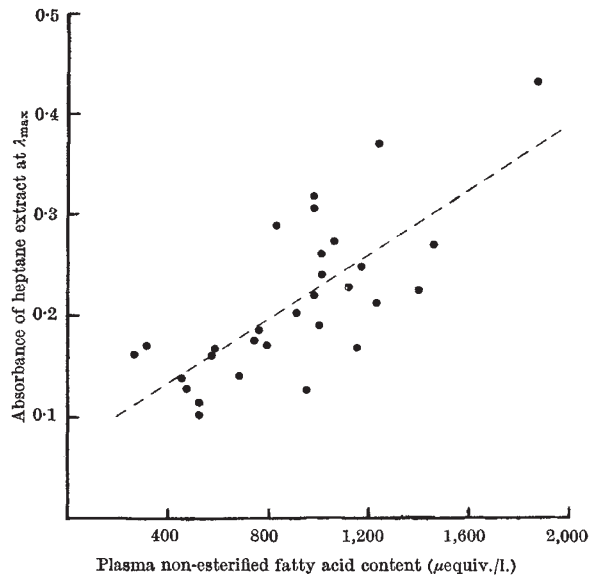

Fig. 1. Relationship between non-esterified fatty acid and carotenoid content of plasma samples obtained from stall-fed cows near to calving. Two ml. plasma were extracted with an acidic heptane-isopropanol mixture by the method of Dole[6]. The heptane extract (8 ml.) so obtained was analysed for long-chain fatty acid content[7] and the absorbance was measured in a 40-mm micro-cell at λ_{max} (446–450 mμ)

Thus it appears that normal physiological increases in the rate of mobilization of depot fat occurring near to calving are associated in the stall-fed cow with increases in plasma carotenoid content. These pigments are largely carotenes, and although in this respect the phenomenon differs from that reported for starving or semi-starving sheep[2,3] the essential similarity is that in either case carotenoids seem to be mobilized from the adipose tissue along with the non-esterified fatty acids.

In view of the general simplicity of the procedure used, it is considered that in sheep and in stall-fed cattle absorbance measurements at 450 mμ or even the appearance of plasma heptane extracts may be a useful alternative to non-esterified fatty acid estimations as a rough index of the rate of depot fat mobilization.

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[1] Patterson, D. S. P., Burns, K. N., Cunningham, N. F., and Saba, N., *J. Agric. Sci.*, **62**, 253 (1964).
[2] Patterson, D. S. P., *Biochem. J.*, **90**, 39P (1964).
[3] Patterson, D. S. P., *J. Agric. Sci.*, **64**, 273 (1965).
[4] Craige, A. H., and Chung, A. C. W., *Proc. Eighty-seventh Ann. Meeting, Ann. Vet. Med. Assoc.*, 166 (1950).
[5] Patterson, D. S. P. (unpublished results).
[6] Dole, V. P., *J. Clin. Invest.*, **35**, 150 (1956).
[7] Patterson, D. S. P., *Res. Vet. Sci.*, **4**, 230 (1963).
[8] Randereth, K., *Thin Layer Chromatography*, 152 (Academic Press, 1963).

PSYCHOLOGY

Generation of a Spiral After-effect by Interaction of Rods and Cones

The well-known spiral after-effect can be generated by looking for some time at a rotating, apparently contracting spiral. When the spiral is stopped it appears to expand outwards. It appears that both rods and cones