

that the affinity of  $C_{18:3}$  acid for cholesteryl esters in the cow ( $C_{18:3}$  CE =  $4.0 \times C_{18:3}$  PL) was two to three times greater than that found in the other herbivores studied here ( $C_{18:3}$  CE =  $1.74 \times C_{18:3}$  PL). Since the concentration of  $C_{18:3}$  acid in plasma lipids is higher in calves grazing pasture than in calves fed hay it is suggested that there are two major factors responsible for the high concentration of  $C_{18:3}$  acid in plasma cholesteryl esters of the cow: (a) preferential esterification of cholesterol with  $C_{18:3}$  fatty acid by the plasma acyl transferase; (b) presence of grass in the diet.

## REFERENCES

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**Absorption of iodinated oleic acid— $^{131}\text{I}$  in rats adapted to diets with different levels of oleic acid.** By G. VARELA and GLORIA URBANO, *Laboratory of Animal Physiology, University of Granada, Spain*

In previous experiments we have seen that the type of fat to which an animal is adapted influences its absorption and that on suddenly changing the type of fat in the diet a significant decrease is produced in its digestibility.

We now study the influence of the adaptation of three groups of rats to diets each containing 10% total fat but with different levels of oleic acid, for a period of 30 days. At the end of this period all the animals were given the same quantity of  $^{131}\text{I}$ -labelled oleic acid (0.5 ml with  $10 \mu\text{c}$ ) by means of a gastric catheter. The level of radioactivity in blood was determined in the animals from this moment and at hourly intervals for the next 8 h with the following results:

Table 1. *Levels of radioactivity in blood (counts/min in total blood)*

Hours	Group 1 26% oleic acid	Group 2 82% oleic acid	Group 3 100% oleic acid
1	42 746 ± 818	58 563 ± 1 209	313 448 ± 5 729
2	58 010 ± 700	102 537 ± 755	355 553 ± 4 721
4	69 632 ± 794	130 687 ± 3 844	464 255 ± 6 608
5½	112 436 ± 656	180 841 ± 3 332	422 840 ± 5 538
6½	95 415 ± 729	277 794 ± 719	760 013 ± 15 395
7	} Descending values with great dispersion Coefficient of variation > 20%		
8			

All the results are the average values, with the mean error for ten animals.

Variance analysis shows that the differences between the three groups are statistically significant at all time intervals.

It is concluded that the higher the level of oleic acid to which the animal is adapted the greater the absorption of  $^{131}\text{I}$ -labelled oleic acid. These results agree

with those obtained by us by the digestibility method and which showed that adaptation to a given fat influenced its digestibility.

**\*The fatty acid composition of adipose and muscle tissue in domestic and free-living ruminants.** By M. M. GALE, M. A. CRAWFORD and M. WOODFORD, *Nuffield Institute of Comparative Medicine, The Zoological Society of London, NW1*

**A folate-protein complex in cow's milk.** By J. E. FORD, D. N. SALTER and K. J. SCOTT, *National Institute for Research in Dairying, Shinfield, Reading*

Folic acid in human blood serum was freely dialysable whereas that in milk was strongly and specifically bound to a minor whey protein. In cow's milk this binding protein was present in excess and the milk had the capacity to bind about 50  $\mu\text{g}$  added folic acid/l. Serum folate levels are relatively very low and the physiological effect of the binding protein is presumably to accumulate folate into the milk against a considerable concentration gradient.

A highly enriched concentrate of the folate-protein (FP) was prepared from rennet whey by ammonium sulphate fractionation. FP precipitated at between 45–60% ammonium sulphate saturation. This fraction was exhaustively dialysed against 0.005 M-phosphate buffer of pH 7.0 containing 0.002 M-2-mercaptoethanol (ME), and chromatographed in a column of DEAE-cellulose. On elution with the 0.005 M-buffer FP was only weakly retarded whereas most of the accompanying protein was retained by the column. Chromatography in DEAE-cellulose was repeated and followed by gel filtration in Sephadex G-150. Sedimentation analysis showed that this preparation was heterogeneous over a narrow molecular weight range around 70 000, with a major component of mol. wt *c.* 73 500. Gel filtration gave a closely similar value, and showed a peak of folate activity at mol. wt 76 000. On starch gel electrophoresis at pH 2.0 in presence of ME and 5 M-urea, FP moved towards the cathode and separated from slower-moving components, probably  $\gamma$ -globulins, and a faster-moving component. At pH 8.6, in absence of urea, a major band containing FP moved slightly towards the anode, and a poorly defined minor band moved towards the cathode. The milk folate was N<sub>5</sub>-methyltetrahydrofolate, as judged by paper chromatography and differential microbiological assay. It was entirely protein-bound at pH 6–8.8; at pH 5 free folate was present and at pH 3.6 the complex was wholly dissociated. On restoring the pH to 7.0 the folate and protein recombined. In 8 M-urea the complex was completely dissociated at pH 6.0. Heating for 10 min at 100° caused irreversible dissociation.

With increasing purity the folate-protein in solution has proved unstable during frozen storage, forming insoluble aggregates of high molecular weight. This has