to that observed (Okuda & Fujii, 1967, 1968) with rat adipose-tissue and liver lipase activity after acetone treatment.

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The Influences of β -Casein Removal on Bovine Casein Micelles

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Over 50% of the β -casein is dissociated from casein micelles (relative α_s -, β - and κ -casein composition 5.6, 3.4 and 1.0 respectively) together with less than 5% of the α_s - and 20% of the κ -casein by gel filtration (Downey & Murphy, 1970) of skim milk at 4°C. Approx. 80% of the casein removed from the micelles and eluted with the whey proteins is β -casein. The average relative composition of the resultant micelles that are eluted at the void volume is 6.4, 2.8 and 0.8 parts of α_s -, β - and κ -casein respectively, and their stability towards Ca^{2+} and susceptibility to rennin proteolysis are similar to those reported (McGann & Pyne, 1960; Fox, 1969) for milk. On a second gel filtration of these micelles a further 30% of the β -, 10% of the κ - and 5% of the α_s -casein is dissociated and the resultant micelles (6.8, 2.2 and 1.0 parts of α_s -, β - and κ -casein respectively) are precipitated by 50mmol per litre of calcium chloride (1h at 37°C). Approx. 10% of the β -casein of these micelles, but very little α_s - or κ -casein, is removed by a third gel filtration, and the micelles (7.5, 1.7 and 0.8 parts of α_s -, β - and κ -casein respectively) are eluted at the void volume and are unstable to 10mmol per litre of calcium chloride (1h at 37°C). On subsequent gel filtration little or no free casein is detected, and the casein micelles eluted as an opaque peak at the void volume (mol.wt. $> 10^8$) attain a final relative composition of 8.0, 1.2 and 0.8 parts of α_s -, β - and κ -casein and are immediately precipitated on addition of 10mmol per litre of calcium chloride at 37°C.

This investigation suggests that hydrophobic interactions are involved in maintaining most (approx. 80%) of the β -casein within the hydrophobic core (Hill & Wake, 1969) of casein micelles. As the temperature is decreased hydrophobic bonds are weakened (Mahler & Cordes, 1967), and this β -casein accordingly dissociates and is released

through the structural lattice (Ribadeau-Dumas & Garnier, 1970) of the casein micelles. As indicated by restricted passage through the pores of Sephadex G-200, the free β -casein exhibits an apparent molecular weight of 200 000, and, since at least 50% of the β -casein is removed without micellar disintegration or affecting stability towards Ca^{2+} , it is suggested either that the β -casein aggregates subsequent to its removal or that some of the pores of casein micelles are sufficiently large to permit diffusion of proteins of this effective molecular size. The decreased stability towards Ca^{2+} after the removal of additional β -casein may have resulted from the concomitant dissociation of κ -casein. The residual β -casein (approx. 20%) appears to be more strongly bound.

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Possible Role of Cytidine Diphosphate Choline in the Complexing of Carbohydrate to β -Lipoprotein in Liver

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Choline derivatives stimulate the synthesis of both the peptide and the carbohydrate moieties of soluble proteins released by rat liver (Mookerjea, 1971; Mookerjea & Marai, 1971). Since [$1\text{-}^{14}\text{C}$]glucosamine is incorporated into low-density lipoproteins (Mookerjea, Jeng & Black, 1967), it was decided to investigate the effect of choline derivatives on the complexing of carbohydrate to this protein fraction and the possible role that the carbohydrate may play in regulating secretion of the macromolecules.

The complexing of carbohydrate to β -lipoprotein was followed by measuring the incorporation of [$1\text{-}^{14}\text{C}$]glucosamine and UDP-*N*-acetyl[$1\text{-}^{14}\text{C}$]glucosamine with rat liver slices and subcellular particles. After dialysis of the incubation media, the β -lipoprotein was precipitated by the Ca^{2+} -heparin method of Jordan, Faulkner & Knoblock (1966). Immunoelectrophoresis of the medium β -lipoprotein showed identity with that

isolated from serum. With liver slices phosphorylcholine caused a marked stimulation of glucosamine incorporation in 2h. To distinguish between synthesis and secretion of the macromolecule, the experiments were next extended to subcellular particles. Significant labelling was observed in both the media and sonic extracts of total microsomal fraction, rough endoplasmic reticulum and Golgi apparatus. The incorporation was dependent on the time and temperature of incubation and on the amount of subcellular particles present. The addition of 2mM-CDP-choline to the cell-free incubation system caused a marked stimulation in incorporation of the label. The β -lipoprotein released by the rough endoplasmic reticulum showed the largest increase in labelling, indicating that the maximum stimulation of glycosylation in response to CDP-choline occurred on the nascent polypeptide and that the presence of the complete lipoprotein molecule was not necessary for activation. The incorporation of the label into total proteins other than β -lipoprotein was also stimulated by CDP-choline, but the specific radioactivity of the β -lipoprotein was considerably higher. When the microsomal fraction was incubated with CDP-[1,2- 14 C]choline under the same conditions used for nucleotide sugar incorporation, phosphorylcholine was markedly incorporated into lecithin (phosphatidylcholine) within 2 min, and after 30 min over 30% of the label presented to the system was found in the lecithin molecule. These results support the concept that the stimulation of *N*-acetylglucosamine complexing into secretory proteins is related to membrane lecithin regeneration.

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Immunochemical Relationships among Plasma and Egg-Yolk Lipoproteins of the Laying Hen

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Selected lipoproteins in hen's plasma and egg yolk have been linked in terms of subunit dis-

sociation and amino acid composition (Bernardi & Cook, 1960) and lipid distribution (Schjeide, 1963). Similarity of plasma and yolk proteins has also been claimed on the basis of electrophoretic mobilities (McCully, Maw & Common, 1959) and immunoprecipitation of serum light lipoprotein and lipovitellin with anti-(egg-yolk-fluid lipoprotein) and anti-(phosvitin-lipovitellin) respectively (Schjeide *et al.* 1963). Since other experiments have shown that serum precursors are split during transfer (Schjeide & Urist, 1959) and that labelling patterns of lipid and protein moieties are different (Schjeide, 1963), a more definitive relationship among the plasma and yolk lipoproteins was sought in the present study.

Rabbit antisera were prepared to very-low-density and high-density egg lipoproteins. Anti-(very-low-density lipoprotein) reacted in agar with total plasma, egg and plasma very-low-density lipoproteins and egg high-density lipoprotein to give single immunoprecipitin lines. Plasma high-density lipoprotein showed no reaction. With anti-(high-density lipoprotein) both very-low-density and high-density lipoproteins from plasma and yolk as well as the total lipoproteins reacted. Egg high-density lipoprotein gave two bands, whereas the others formed only one. Partial delipidation with diethyl ether produced plasma and yolk very-low-density lipoproteins that reacted with anti-(very-low-density lipoprotein) to give one heavy and one light band. Total plasma and yolk each gave three bands, and high-density lipoproteins from both sources gave single bands with anti-(very-low-density lipoprotein) after delipidation. Immunoelectrophoresis showed that both very-low-density lipoproteins contained two proteins that reacted with anti-(very-low-density lipoprotein). Egg very-low-density lipoprotein gave two arcs with anti-(high-density lipoprotein), whereas plasma very-low-density lipoprotein gave only one. Both high-density lipoproteins gave single arcs with anti-(high-density lipoprotein). Total plasma and yolk gave three arcs with anti-(very-low-density lipoprotein) and at least two against anti-(high-density lipoprotein).

The results show that plasma and egg very-low-density lipoproteins and high-density lipoproteins are immunochemically identical. The very-low-density lipoprotein cross-reacts with anti-(high-density lipoprotein), suggesting that the plasma very-low-density lipoprotein contains antigenic determinants of both very-low-density and high-density lipoproteins from yolk. Native egg very-low-density lipoprotein and high-density lipoprotein are sufficiently open to precipitate either anti-(very-low-density lipoprotein) or anti-(high-density lipoprotein), and delipidation has little effect. The plasma high-density lipoprotein contains at least