75000) are totally excluded from the mitochondrion, certain smaller molecules such as sucrose penetrate only as far as the inner membrane and the total water space may be measured with tritiated water. Using these spaces as markers, we have investigated the distribution volumes for (-)-carnitine and acetyl(-)-carnitine in rat liver mitochondria incubated at 0°C for 3 min at pH 7.2 in a medium consisting of tris-HCl buffer (20 mM), potassium chloride (80 mM), EDTA (1 mM) and sucrose (20 mM). After incubation mitochondria were obtained by centrifugation at 12000g for 10 min. In these experiments the total pellet water averaged 2.22 µl/mg of protein and the [¹⁴C]sucrose and [¹⁴C]dextran spaces were 68% and 38% respectively of the total pellet water. The penetration of tritiated (-)-carnitine was identical with that of sucrose. However, values for the acetyl(-)-carnitine space, measured enzymically, were quite erratic and occasionally less even than the dextran space. This anomaly was traced to a breakdown of acetyl(-)-carnitine during the incubation and subsequent manipulations. Experiments were therefore repeated, but the technique of high-speed centrifugation of mitochondria through a silicone layer into perchloric acid was employed to give a near-instantaneous deproteinization of the mitochondria after separation from the incubation medium. In this system the acetyl(-)-carnitine space was also found to be identical with the sucrose space. In parallel experiments the addition of acetyl(-)-carnitine was observed to elevate the intramitochondrial acetyl-CoA concentration from 0.88 to 1.4 nmol/mg of protein. This suggests that acetyl groups enter the mitochondrial matrix under conditions in which acetyl(-)-carnitine does not penetrate the inner membrane.

Results reported suggest the concept of an anisotropic mechanism for carnitine facilitation of fatty acyl group translocation (Haddock, Yates & Garland, 1970), provided that two assumptions are valid. First, acetyl(-)-carnitine must not have been rapidly removed as soon as it entered the matrix. Secondly, it is assumed that discrete subcompartments of the mitochondrial matrix do not exist. If there were compartmentation within mitochondria, small changes in accumulation of acetyl(-)-carnitine might well be non-detectable. The validity of these assumptions will be discussed.

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Lipase Activity of Bovine Mammary Gland

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The lipase activity of the mammary-gland extracts (0.4–1.5 µequiv. of acid/min per g of tissue; substrate tributyrin emulsion) is associated with macromolecules. These are not sedimented by centrifugation at 38000g for 2 h and are eluted at the void volume of Sepharose 4B columns, indicating an apparent molecular weight greater than 20×10^6 . On mixing pancreatic lipase with the extracts the enzyme binds to the macromolecules, which thus appear to be complexes composed of mammary-gland lipase(s) in association with other proteins. Lipid or other hydrophobic interactions are apparently involved in maintaining the mammary-gland lipase complexes; the addition of dimethylformamide (final concn. 1.3 M) to the extract followed by gel filtration on Sepharose 4B columns equilibrated with eluent containing dimethylformamide dissociates the complexes, and the lipase activity is eluted (recovery 60%) in a position corresponding to a protein of molecular weight 0.63×10^6 . Similarly the complexes are also completely dissociated by gel filtration on Sepharose 2B in the absence of dimethylformamide and, since the eluted lipase again has an apparent molecular weight of approximately half a million, it may represent the free enzyme.

Milk lipases may be derived from the mammary gland and the casein-lipase complexes of milk (mol.wt. $> 100 \times 10^6$) exhibit a number of properties (Downey & Murphy, 1970) similar to those of the mammary-gland activity.

In the absence of dimethylformamide the lipase activity is quantitatively eluted from Sepharose 4B columns, which had been calibrated for molecular-weight determination with spherical plant viruses and macroproteins, as two overlapping peaks at or near the void volume (mol.wt. $> 20 \times 10^6$) followed by a third lipase peak (mol.wt. approx. 1.5×10^6). The first peak appears to represent the intact lipase-protein complexes and exhibits little esterase activity. The other two are attributed to intermediate dissociation products, and the esterase activity of the extracts (0.4–0.8 µequiv. of acid/min per g of tissue; substrate *p*-nitrophenyl acetate) is quantitatively eluted with the latter of these two peaks. After dimethylformamide treatment the esterase is eluted (recovery 60%) coincidentally with the free lipase in a single peak of molecular weight 0.63×10^6 . These results may indicate an alteration in the substrate specificity of the lipase resulting from dissociation of the lipase-protein complexes, similar

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