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

two antigenic determinants, both of the egg high-density-lipoprotein type, one of which is common to the very-low-density lipoprotein. In native high-density lipoprotein only the unique determinant is sufficiently exposed to react. On delipidation high-density lipoprotein also reacts with anti-(very-low-density lipoprotein). The difference between very-low-density lipoprotein and high-density lipoprotein is apparently due to the lipid moiety of the yolk high-density lipoprotein.

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An Alkaline Proteinase in Muscle Homogenates

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Homogenates of mouse or rat skeletal muscle in 50mM-potassium chloride degraded casein to perchloric-acid-soluble products at pH 8.3. The products were measured by extinction at 224nm, and at 260nm to correct for nucleic acid breakdown (which was relatively small). After centrifuging of the homogenates at 100000g for 30min nearly all of this proteolytic activity was usually found in the sediment fraction. The sedimented activity, when measured over the range pH 7.5–12.1, was maximum at pH 11; it was increased by 0.3M-sodium chloride, slightly at pH 10 but four- to five-fold at pH 7.5. The activity was lost when the fraction was heated at 80°C for 10min. Soya-bean trypsin inhibitor (1µg/0.3ml) produced a 77% inhibition of activity; there was no inhibition by hen-ovomucoid trypsin inhibitor (1µg) or by the supernatant (0.05ml) obtained by centrifuging a dialysed muscle homogenate at 100000g for 30min. Mouse serum (diluted 1:200; 0.05ml) caused a slight (10%) inhibition.

Attempts were made to solubilize the enzyme by extracting the fraction for 15min at room

temperature with various reagents followed by centrifuging at 30000g for 30min. Little or no enzyme was extracted with tricine buffer, pH 8.0 (30mM), acetate buffer, pH 5.0 (30mM), Triton X-100 (0.3%) or urea (8M)+2-mercaptoethanol (10mM). Sodium dodecyl sulphate (10mM) or guanidine hydrochloride (5M)+2-mercaptoethanol (10mM) completely inactivated the enzyme. The enzyme was extracted with 0.75M-sodium chloride or -potassium thiocyanate after removal of a large part of the inactive protein (mainly myofibril protein) with 8M-urea. The enzyme was slowly precipitated on standing in the cold and could be redissolved in 0.75M-potassium thiocyanate resulting in a tenfold purification (460-fold compared with the original homogenate). The extract hydrolysed *N*-benzyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester but not *N*-toluene-*p*-sulphonyl-L-arginine methyl ester.

Huston & Krebs (1968) purified a Ca²⁺-dependent proteolytic enzyme from muscle extracts, with maximum activity at pH 7.5. Brush & Cheng (1968) purified an insulin-degrading protein from rat muscle, also with optimum activity at pH 7.5. Neither of these enzymes appears to be identical with the one studied here. The autolytic breakdown of a muscle myofibril fraction reported by Noguchi & Kandatsu (1969, 1970) also appears to be due to a different enzyme, since it is strongly inhibited by the supernatant fraction.

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The Effect of Chlorambucil on Nuclear Protein Synthesis in Yoshida Ascites-Sarcoma Cells

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Increases have been reported in the nucleic acids and protein content of a drug-sensitive strain of Yoshida ascites cells after treatment of the host animals with alkylating drugs; comparable changes have not been observed in a drug-resistant strain of the tumour (Harrap & Hill, 1969a,b). After the administration of a therapeuti-