and freeze-dried *in vacuo*, and lipid was extracted in acetone, chloroform-methanol (2:1, v/v) and light petroleum. Normal lenses from individuals of similar age were obtained *post mortem* and used as controls. The proteins from each type of lens, obtained in this way, were investigated by gel filtration of 6% agarose (Bio-Gel A-5m; Bio-Rad Laboratories, Richmond, Calif., U.S.A.) under highly dissociating conditions, either as reduced protein-sodium dodecyl sulphate complexes in 0.1% sodium dodecyl sulphate, or in 6M-guanidinium chloride after reduction and carboxymethylation (Fish, Mann & Tanford, 1969; Fish, Reynolds & Tanford, 1970). Essentially similar behaviour was observed in each dissociating medium. The main bulk of the protein emerged as a broad peak with K_d 0.54 in sodium dodecyl sulphate or K_d 0.38 in guanidinium chloride. With proteins from group III cataractous lenses an earlier peak (K_d in the range 0-0.12) was observed that was absent from normal control lenses and accounted for 15-20% of the applied protein. This indicates that a significant proportion of the polypeptide chains from these cataractous lenses was present as aggregates with a molecular weight above 5×10^4 , maintained by covalent bonds that are not disulphide linkages. A similar conclusion was reached by electrophoresis of the reduced protein-sodium dodecyl sulphate complexes in polyacrylamide gel, essentially by the method of Weber & Osborn (1969).

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Characterization of Components of Inhibitory-Factor (Troponin B) Preparations of the Myofibril

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Inhibitory-factor preparations from rabbit skeletal muscle contain, in addition to the component of molecular weight 23000 reported by Schaub & Perry (1971), two other proteins whose molecular weights were determined by electrophoresis in sodium dodecyl sulphate (Weber & Osborn, 1969) to be 37000 and 14000 respectively.

Preparations obtained from troponin (Schaub &

Perry, 1971) by an acid precipitation procedure similar to that described by Hartshorne & Mueller (1968) contained the components of molecular weight 37000 and 23000. On polyacrylamide-gel electrophoresis in 6M-urea at pH 3.2 each protein migrated as a sharp band, although occasionally the component of molecular weight 23000 migrated as two bands of similar mobility. After carboxymethylation the component of molecular weight 23000 migrated as a single band.

Inhibitory factor prepared by the dissociation of troponin on SE-Sephadex (sulphoethyl-Sephadex) in 6M-urea (Schaub & Perry, 1969) also contained the components of molecular weight 37000 and 23000. In these preparations the latter usually migrated as a multiple band system on electrophoresis at pH 3.2. This difference between the two preparations disappeared on electrophoresis in sodium dodecyl sulphate after reduction with β -mercaptoethanol. In addition, the preparations obtained by dissociation in urea contained the component of molecular weight 14000. The relative amounts of this component present in different preparations varied widely, for reasons as yet not understood.

When separated from the component of molecular weight 37000 by chromatography on CM-cellulose, 15-20 μ g of the component of molecular weight 23000 produced 50% of the maximum inhibition of the Mg^{2+} -stimulated adenosine triphosphatase activity of 1 mg of desensitized actomyosin in the presence of tropomyosin. The component of molecular weight 37000 was not active as an inhibitor. It replaced neither tropomyosin in enhancing the inhibitory action of the component of molecular weight 23000 nor the calcium-sensitizing factor in the relaxing-protein system. The component of molecular weight 14000, separated from the other components by gel filtration on Sephadex G-100, also possessed inhibitory activity, which preliminary results suggest was not enhanced by tropomyosin, as was the case with the component of molecular weight 23000.

The components of molecular weight 37000 and 23000 were present in whole myofibrils and in all troponin preparations examined. Troponin preparations contained variable amounts of the component of molecular weight 14000, and the possibility that it is derived from the component of molecular weight 23000 is currently under investigation. All three components had distinctive amino acid compositions, particularly in their cysteine content, although broad similarities between them could be recognized.

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Lipid Metabolism in Experimental Cholestasis

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Disturbances in lipid metabolism have been reported in human and experimental obstructive jaundice. Thus human studies have shown elevated concentrations of plasma free fatty acids (Mortiaux & Dawson, 1961), serum triglycerides (Mendenhall & Mortiaux, 1961) and cholesterol (Man, Kartin, Durlacher & Peters, 1945), whereas in animal experiments elevated concentrations of cholesterol (Byers, Friedman & Michaelis, 1951) and abolition of the negative-feedback regulation of cholesterol biosynthesis (Katterman & Creutzfeldt, 1970) have been described. The purpose of the present investigation was to study lipid and ketone-body metabolism in the rat during experimental cholestasis.

Bile-duct ligation or sham operations were performed on anaesthetized rats and the animals were killed 6–9 days later. All rats were starved for 48h before being killed. Blood was collected from one group for determinations of triglycerides, free fatty acids, cholesterol and bilirubin and enzymic analysis of blood metabolites. The mean bilirubin concentration in the ligated animals was 9mg/100ml.

Another group of animals were killed by cervical dislocation, and the livers were freeze-clamped between clamps cooled in liquid N₂ and metabolites were determined in the deproteinized liver extracts by enzymic methods.

The most striking change noted was a fall in blood and liver ketone-body concentrations to 31 and 28% respectively compared with the corresponding values for the sham-operated rats after 9 days of biliary stasis. This was accompanied by a 73% decrease in hepatic acetyl-CoA concentration. The [3-hydroxybutyrate]/[acetoacetate] ratio fell from 2.7 to 1.1 and this was associated with a fall in the [glutamate]/[2-oxoglutarate][NH₄⁺] ratio consistent with a more oxidized mitochondrial [free NAD⁺]/[free NADH] ratio (Williamson, Lund & Krebs, 1967). The [lactate]/[pyruvate] ratios remained unchanged. Concentrations of plasma free fatty acids were normal or only slightly lower than in the controls, whereas those of plasma triglycerides and serum cholesterol were increased (146 and 200% respectively).

These results suggest a decrease in the β -oxidation of fatty acids and a stimulation of cholesterol and triglyceride synthesis. Possible mechanisms for these metabolic alterations will be discussed.

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An Explanation for the Lowering of Blood Ketone-Body Concentrations in Starved Rats during Short-Term Exercise

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The fall in blood ketone-body concentrations (45%) in starved rats during short-term exercise was ascribed by Drury, Wick & MacKay (1941) to increased peripheral utilization of ketone bodies. However, electrical stimulation of isolated perfused rat skeletal muscle does not increase ketone-body uptake (Houghton & Ruderman, 1970). Short-term severe exercise is also characterized by a rise in circulating lactate concentration (up to about 10mM), and as lactate can have an anti-lipolytic action (Bjorntrop, 1966) the effect of infusion of L(+)-lactic acid into resting starved (48h) rats was investigated. A concentration of about 7mM was reached in 10min and maintained for a further 20min. Blood ketone-body concentrations fell from 4.51 ± 1.51 mM (mean $\pm$ S.D., five observations) to 2.29 ± 0.55 mM by 10min and to 1.03 ± 0.41 mM at 30min. There was a concomitant decrease in the plasma free fatty acid concentration from 1.40 ± 0.23 μ equiv./ml to 0.74 ± 0.18 μ equiv./ml after 30min infusion. On cessation of infusion there was a rise in the free fatty acid and ketone-body concentrations.

The results suggest that the fall in the concentration of blood ketone bodies during short-term exercise is mainly due to an inhibition of free fatty acid release from adipose tissue and perhaps also to a direct anti-ketogenic effect of lactate on the liver.

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