contained 69% of the applied thiol. Some RNA and a discrete peak of low-molecular-weight thiol compounds were retained in the gel. The thiol-containing protein peak was further fractionated on microgranular DEAE-cellulose giving seven components. About 40% passed through the column unabsorbed and, apart from 8M-urea, this protein could only be dissolved in 1M-acetic acid. When the latter solution was passed through a CM-cellulose column, quantitative absorption occurred. The main bulk of the thiol material could be eluted with 20mM-HCl, giving a fraction containing 78nmol of SH/mg. of protein. Analysis gave a ratio of Asp + Glu : Lys + Arg + His of 2:1.

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Effects of Trypan Blue on Rat Liver Heterolysosomes

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The effect of intralysosomal trypan blue on the digestive capacity of rat liver heterolysosomes has been studied, the experimental methods being based on those described by Mego, Bertini & McQueen (1967). Bovine serum albumin was labelled with ^{125}I by the method of McConaghey & Dixon (1966) and denatured as described by Mego *et al.* (1967). Male Wistar rats (275–310g.) were injected intravenously with 1.4mg. of protein/kg. body wt. and killed, and their livers were perfused with cold 0.9% NaCl. After homogenization in cold 0.25M-sucrose a pellet sedimenting between 11000g-min. and 330000g-min. was prepared by centrifugation. This pellet was suspended in tris-acetic acid buffer, pH 7.4, containing 0.25M-sucrose and incubated at 37°, and samples were withdrawn at intervals up to 2hr. Each sample was assayed for trichloroacetic acid-soluble radioactivity (a measure of the extent of proteolysis) and for non-sedimentable trichloroacetic acid-insoluble radioactivity (a measure of free albumin and hence of heterolysosome rupture).

Acid-soluble radioactivity increased from 10% to 36–41% of the total radioactivity over 2hr.; no increase was observed when 0.2% of Triton X-100 was included in the incubate, indicating that proteolysis occurs within osmotically active particles. Free albumin increased from 4% to 25–35%

in the first 30min. and remained at this value for the duration of the incubation. Inclusion of 0.2% of Triton X-100 in the incubate caused the immediate release of 80–90% of the albumin.

Rats that had received trypan blue (75mg./kg.) subcutaneously 24hr. before injection with albumin consistently showed a lower rate of protein breakdown than controls, trichloroacetic acid-soluble radioactivity reaching only 20% after 2hr. This decreased rate of proteolysis was accompanied by an increased release of free albumin, which reached 50% of the total radioactivity within 30min. This effect of intralysosomal trypan blue on heterolysosome stability probably explains the lower rate of protein breakdown observed in incubates of particles from dye-treated rats. However, since trypan blue inhibits lysosomal enzymes (Lloyd, Beck, Griffiths & Parry, 1968), intralysosomal enzyme inhibition may also contribute to the decreased rate of protein breakdown.

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Pyruvate Carboxylase in Lactating Rat and Rabbit Mammary Gland

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Though the role of pyruvate carboxylase (EC 6.4.1.1) in gluconeogenesis is well established, it has been postulated (Wise & Ball, 1964; Ballard & Hanson, 1967) that in adipose and liver the enzyme has a role in lipogenesis. Lactating mammary gland has a high lipogenic activity (Fritz, 1961; Masoro, 1962), yet the presence of pyruvate carboxylase has not been reported in this tissue. We have therefore investigated some properties of this enzyme in lactating rat and rabbit mammary gland.

Preliminary experiments indicated that pyruvate carboxylase was present in both the mitochondrial and particle-free fractions from homogenates of rat mammary gland, but confined to the mitochondrial fraction of homogenate of rabbit mammary gland. Optimum conditions for the assay of this enzyme in these fractions have been determined. The cofactor requirements do not differ significantly