7 and 4, and at 18°C and 0°C. Solution was measured spectrophotometrically at 530nm. The stability of the solutions was tested over 12h.

Degradation of the glycoproteins of the membrane was studied by exposing samples of purified membrane glycoprotein (Stoddart & Metcalfe, 1969) to the solvents and examining the changes in their electrophoretic patterns (Takayama, MacLennan, Tzagoloff & Stoner, 1966) and composition. The extent of the dissociation of both membranes and purified protein was studied by analytical ultracentrifugation.

It was found that pyridine, the picolines and 2,6-lutidine were good solvents at 30% (v/v) above pH 8.7 (as confirmed by ultracentrifugation) but not below this pH. At 18°C there was degradation of sialoglycoprotein, but this was stable up to pH 9.7 at 0°C. Piperidine and pyrrolidine were effective solvents only at pH values far above 10. *N*-Methylpyrrolidone (90%, v/v) was a good solvent at pH 7.

No esters or glycols were found to be good solvents. 2-Mercaptoethanol (90%, v/v) dissolved membranes at pH 7, and 2-chloroethanol was fairly effective at 90% (v/v), at 60% (v/v) at pH 4 and at 30% (v/v) at pH 9. 2,2-Dichloroethanol was a good solvent above 50% (v/v) at all pH values and dissociated the protein very extensively.

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Reversible Dissociation of Fatty Acid Synthetase from Lactating Rat Mammary Gland

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The native fatty acid synthetase isolated from lactating rat mammary gland and maintained at 21°C exists as a 13S species. Aging at 0°C for 1 week, in either the presence or the absence of dithiothreitol, results in complete dissociation into half-molecular-weight 9S subunits (Smith & Abraham, 1970). Synthetase activity could be demonstrated with the 9S species formed by aging in the presence of dithiothreitol even after removal of the dithiothreitol. However, no activity was observed with the 9S species formed by aging in the absence of dithiothreitol.

The question whether both native and subunit species possess synthetase activity or whether reassociation occurs under conditions of the enzyme assay was investigated by studying the effect of temperature on enzyme activity and structural integrity of the 9S and 13S species. The temperature-dependence of fatty acid synthetase activity was markedly different when 9S and 13S species were added to the assay medium. Enzyme activity was observed with the 13S species at temperatures as low as 0°C, but no significant activity could be detected below 20°C with the 9S species. Between 20°C and 30°C the activity observed with the 9S species was very much more temperature-dependent than that with the 13S species. The 9S species was incubated in the absence of dithiothreitol for 3 min at 20°C and at 30°C and analysed by sucrose-density-gradient centrifugation at 2°C. Whereas the original 9S preparation contained only 6% of the 13S species, the samples incubated at 20°C and 30°C contained 22% and 41% respectively of the 13S species. Thus, whereas the cold-induced dissociation of the native enzyme is a slow process occurring over a period of days, the heat-induced reassociation is extremely rapid, taking place in a matter of minutes. These findings indicate that the native 13S species is the only enzymically active form of fatty acid synthetase and that the activity observed with the 9S species results from heat-induced reassociation to the active 13S form during assay.

When the 13S species was titrated with *p*-chloromercuri[¹⁴C]benzoate at 20°C, 30% of the enzyme dissociated into the 9S species when about six of the enzyme's 57 thiol groups were blocked. The *p*-chloromercuri[¹⁴C]benzoate-labelled 13S species retained approx. 70% of its enzyme activity, whereas no enzyme activity could be demonstrated

with the *p*-chloromercuri[¹⁴C]benzoate-labelled 9S species formed by dissociation. The *p*-chloromercuri[¹⁴C]benzoate-labelled 9S species cannot undergo heat-induced reassociation to the 13S form and gives no enzyme activity in the fatty acid synthetase assay. This result is consistent with the conclusion that the native 13S species is the only active form of fatty acid synthetase.

Thus the non-covalent forces required for holding together the two subunit halves of fatty acid syn-

thetase are dependent on the maintenance of certain protein thiol groups.

The subunits of rat mammary-gland fatty acid synthetase differ from those of the pigeon liver enzyme, which do not undergo rapid heat-induced reassociation in the absence of a reduced thiol (Butterworth, Yang, Bock & Porter, 1967).

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