bile flow or on liver ATP concentration. Liver and blood analyses indicated that 2,4-dinitrophenol was not entering the liver in significant amounts, probably as a result of a tight binding to plasma albumin (Weinbach, Garbus & Claggett, 1966; Schimassek, 1968). Displacement from the albumin site was attempted by using anionic competitors such as ethyl α -(4-chlorophenoxy)- α -methylpropionate and sodium salicylate. The subsequent effects on bile flow with ethyl α -(4-chlorophenoxy)- α -methylpropionate as the displacing agent were inconsistent and were accompanied by a high mortality rate. Displacement of 2,4-dinitrophenol (20mg./kg. body wt.) by sodium salicylate (60mg./kg. body wt.) gave a decreased bile flow of 27% in the first hour and 12% in the second hour compared with the salicylate controls. At the end of the second hour the liver ATP concentration had decreased to 58% of the control value. As the maximal depression of bile flow occurred 30–45 min. after the injection of 2,4-dinitrophenol plus sodium salicylate, changes in total homogenate and supernatant ATP concentration were investigated at 30 min. after dosing, by using the micro filtration procedure of Delaney & Slater (1967). The results showed that the percentage of total homogenate ATP in the cell-sap fraction was not affected by treatment with 2,4-dinitrophenol plus salicylate, although total homogenate ATP and the total ATP content in the cell-sap fraction of the whole liver were decreased.

The intraperitoneal injection of 40m-moles of fructose/kg. body wt. after 24hr. starvation has been reported to produce a rapid depression of liver ATP concentration (Burch, Max, Chyn & Lowry, 1969). By using this dose it was found that a drop in bile flow of 46% occurred in the first hour and 37% in the second hour after injection; there was a decrease of 40% in total liver ATP by the end of the second hour. These results will be discussed in terms of the role of liver ATP in bile-flow mechanisms.

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Multiple Electrophoresis Peaks of Rat Liver Decarboxylases for 3,4-Dihydroxyphenylalanine and 5-Hydroxytryptophan

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Evidence is accumulating that catecholamines and indolylalkylamines are involved in the development of certain abnormal mental states. For example, Bunney *et al.* (1969) suggest that there are two types of 'depression', biochemically characterized by deficiency of either catecholamines or indolylalkylamines and possibly corresponding to the clinical states of retarded (inhibited) and agitated (psychotic) depression respectively.

The enzymic activities leading to the formation of these amines, 3,4-dihydroxy-L-phenylalanine (DOPA) decarboxylase and 5-hydroxy-L-tryptophan decarboxylase have been given different EC numbers (4.1.1.26 and 4.1.1.28 respectively), but have been reported as associated with a single protein (e.g. from rabbit kidney cortex; Rosengren, 1960). On the other hand, Marmaras, Sekeris & Karlson (1966) have shown simultaneous induction of 3,4-dihydroxyphenylalanine decarboxylase and repression of 5-hydroxytryptophan decarboxylase activities by ecdysone in insect larvae.

Before the significance of catecholamine and indolylalkylamine concentrations in human depression can be evaluated, the nature of the decarboxylase systems that produce them in various mammalian tissues must be clarified.

A sensitive radioisotopic assay for the decarboxylases has been developed in this laboratory, based on the procedure of Karlson & Sekeris (1962), with 3,4-dihydroxyphenyl[2-¹⁴C]alanine and 5-hydroxy-[methylene-¹⁴C]tryptophan. The products are separated from the substrates by one-dimensional paper chromatography, and the radioactivity on the paper is measured by liquid-scintillation counting, the paper being placed directly into toluene scintillator.

Male rat liver homogenate was centrifuged at 10⁵g for 60 min. In this supernatant there was a marked pH-dependence of enzymic activity, with 3,4-dihydroxyphenylalanine decarboxylase activity maximal at pH 6.8 and 5-hydroxytryptophan decarboxylase activity maximal at pH 7.8, in agreement with other workers (Reid, Riley & Shepherd, 1963).

The supernatant was subjected to electrophoresis by the preparative technique of Brownstone (1969), with a 5% total monomer polyacrylamide gel at pH 8.7 in glycine-tris buffer, and with a potential difference of 170 v across a 45 mm.-thick gel. Four peaks of decarboxylase activity were recovered.

These peaks had elution times of 1, 4, 7, and 10 hr., and all four showed decarboxylase activity towards both 3,4-dihydroxyphenylalanine and 5-hydroxytryptophan. There may be slightly greater activity towards 5-hydroxytryptophan in the first fraction, and towards 3,4-dihydroxyphenylalanine in the last two fractions.

The nature and kinetics of the activities represented by these peaks are at present under investigation.

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A Reduced Ovomucin-Reduced Lysozyme Complex from Egg White

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Crude insoluble ovomucin precipitates obtained from the thick fraction of egg white can be purified by extraction of the precipitates with solutions of KCl (Brooks & Hale, 1961; Robinson & Monsey, 1966). This communication describes a preliminary characterization of an insoluble protein fraction that was formed during extensive dialysis of the KCl extracts against distilled water.

The precipitate obtained after dialysis of the KCl extracts was washed with distilled water, dried and dissolved by reduction with 0.3M-mercaptoethanol and subsequent alkylation with 0.36M-iodoacetic acid in 6M-guanidinium chloride at pH 8.2. The elution pattern from a column (50 cm. $\times$ 2.1 cm.) of Sephadex G-100 of the reduced protein fraction showed that all the sample was eluted as one peak at the void volume of the column in the presence of 0.5–2.0M-KCl. However, gel filtration in 6M-urea gave two fractions (A and B).

Fraction A, like preparations of purified reduced ovomucin (Robinson & Monsey, 1966), was eluted at the void volume of a Sephadex G-200 column and contained hexose sugar, mainly galactose (8.9%, w/w), glucosamine (10%, w/w), galactosamine (1.7%, w/w) and N-acetylneuraminic acid (3.9%,

w/w). Fraction B had the same retention volume as reduced-alkylated lysozyme. Artificial mixtures of reduced-alkylated lysozyme and natural non-reduced lysozyme could be fractionated on a Sephadex G-75 column (5 cm. $\times$ 2.1 cm.) in the presence of 6M-urea. When artificial mixtures of 20 mg. of purified ovomucin and 10 mg. of pure lysozyme were reduced and alkylated the reduced ovomucin and reduced lysozyme derivatives could only be fractionated on Sephadex columns in the presence of urea, whereas reduced-alkylated ovomucin and unreduced pure lysozyme could be fractionated on the Sephadex columns in 0.5M-KCl. Amino acid analysis, analytical ultracentrifugation in 2M-guanidinium chloride and electrophoresis on cellulose acetate strips has proved that fraction B was reduced-alkylated lysozyme.

These studies have shown that: (1) the KCl extracts obtained during purification of crude ovomucin contained a dissolved substance that possessed the same constituent carbohydrates as ovomucin (Robinson & Monsey, 1966) and that formed a complex with lysozyme at low ionic strength; (2) reduced-alkylated lysozyme associated more strongly with reduced-alkylated ovomucin, probably through hydrogen-bonding, than did non-reduced lysozyme.

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A Quantitative Study of a Reduced Ovomucin-Natural Lysozyme Interaction

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To understand the interactions of egg-white lysozyme and ovomucin and determine their suggested effect on the rigidity of the thick egg-white gel (Hawthorne, 1950; Cotterill & Winter, 1955; Brooks & Hale, 1959; Garibaldi, Donovan, Davis & Cimino, 1968), it is essential first to determine the stoichiometric composition of a pure lysozyme-ovomucin complex.

Although the preceding communication has shown that reduced-alkylated egg-white lysozyme associated more strongly with reduced-alkylated ovomucin, probably through hydrogen bonds, than did non-reduced lysozyme, it was also shown that natural lysozyme and a KCl-soluble substance similar to ovomucin did form an insoluble complex at low ionic strength. Because natural lysozyme, unlike reduced lysozyme, is soluble in dilute salt solutions and because purified preparations of