THE ABSENCE OF CORTISONE EFFECT ON THE SYNTHESIS OF SPECIFIC HISTONES AND RIBOSOMAL PROTEIN SUBUNITS IN LIVER

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SUMMARY

Possible a priori mechanisms for glucocorticoid mediated hepatic enzyme induction include an alteration of the rate of synthesis of: (a) a histone repressor which may regulate specific gene transcription or (b) a ribosomal "regulatory" protein, or (c) a specific class of ribosomes concerned with the synthesis of the inducible enzymes. Following incorporation of double labelled amino acids into control and cortisone treated animals hepatic histone and ribosomal protein subunits were fractionated by acrylamide gel electrophoresis and the hormonal effects upon their radioactivity evaluated. The glucocorticoids did not detectably influence the synthetic rate of either any specific histone fraction or of any specific ribosomal protein subunits.

Cortisone administration to rats results in a stimulation of the rate of incorporation of radioactive precursors into liver ribonucleic acids as well as an increased rate of synthesis of several specific enzymes (1, 2). The biochemical processes underlying these effects are unclear and whether the primary target of the hormone is upon transcriptional or translational processes remains uncertain. The present experiments were designed to explore the possibility that a specific hormonal effect upon either the turnover of a histone protein or a "regulatory" ribosomal protein occurred which might alter the rates of RNA and protein synthesis and trigger a more general metabolic change.

Histones have been proposed as regulators of gene expression (3). One possible mechanism for selective gene activation during hormonal enzyme induction would be an inhibited rate of synthesis of such a histone "repressor." A double labelling technique was employed to explore this possibility. Six intact fasted Holtzman rats were injected either with L-leucine 4, 5 H^3 (specific activity 29.1 c/mM)(100 μC/100 g body weight) and cortisone acetate (5 mg/100 g body weight) or with L-leucine u. l. C^{14} (specific activity 240 mC/mM) (30 μC/100 g body weight) and saline. Three hours later, the twelve animals were sacrificed and an equal weight of liver from each group was pooled, homogenized and the nuclei isolated according to Piha et al. (4). Histones were extracted with 0.25 N HCl and dialyzed against 1 mM sodium acetate buffer pH 4.5, containing 6 M urea (LeBoy et al., 6). Four major and several minor histones fractions can be obtained with this procedure (Figure 1). It is of interest to note that the histone subunits have differing turnover rates for the different fractions do not have the same specific activity; however, in all fractions, the ratio C^{14}:H^3 was found to be essentially identical (Figure 1), indicating that glucocorticoid hormones do not uniquely influence the synthetic rate of any specific histone fraction.

Although less is known about the possible regulatory function of the structural ribosomal proteins, Kurihara and Wool (7) have shown that insulin, added in vitro to an incubated diaphragm preparation, specifically enhanced the synthesis of several ribosomal proteins. A double labelling procedure similar to that described for the histones was herein employed to assess the effect of glucocorticoid hormones on the turnover of each of the liver ribosomal proteins. In order to maximize radioactivity in the ribosomal proteins, mice were used instead of rats. C57 mice were treated

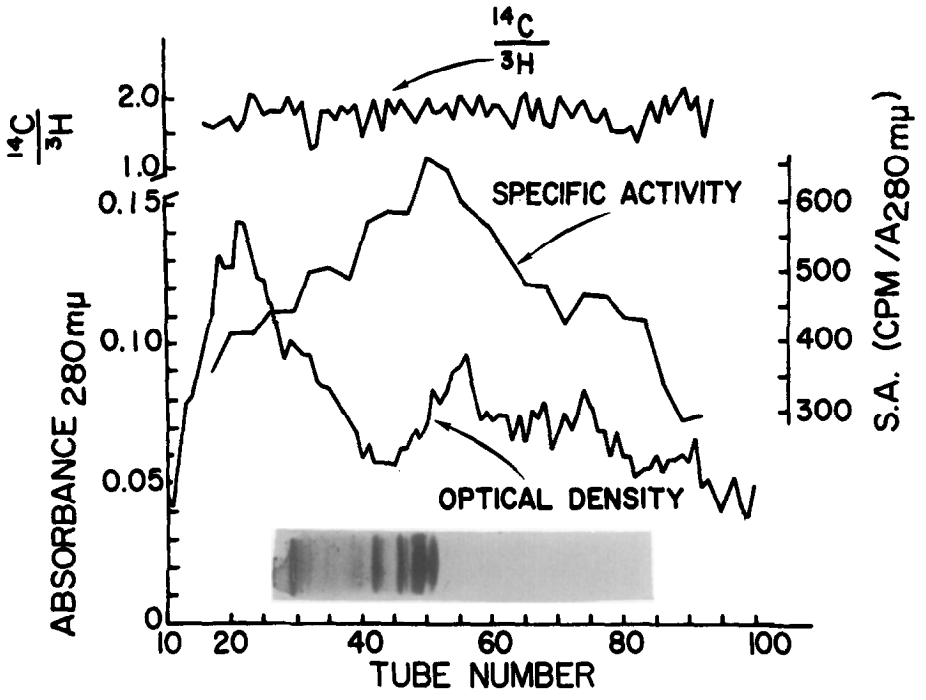

Figure 1: The effects of cortisone upon the incorporation of radioactive amino acid into rat liver histone subunits

25 mg of histones in 1 mM sodium acetate buffer, pH 4.5, containing 6 M urea, are layered onto a 5 x 10 cm preparative 7.5% acrylamide gel column (Buchler Co.); electrophoresis was carried out at 4° C for 22 hours at 50 mA and 320 volts. Fractions of 4 ml were collected at flow rate of 60 ml/hour and the optical density at 280 mμ was monitored; 2 ml aliquots of each fraction were combined with 1 ml of NCS reagent (Nuclear Chicago) and 20 ml of Bray's solution and counted in a Tri Carb Scintillation counter. The counts were corrected for background and quenching and the C^{14}:H^{3} ratio was calculated for each sample. The depicted specific activity refers to the counts per minute in the C^{14} channel per unit $O.D._{280}$. Also shown is an Amido Black stained analytical disc electrophoretic separation of these histones.

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either by cortisone (5 mg/100 g body weight) or saline. Four hours later, the former was injected with leucine-C^{14} (600 μC/100 g body weight), the latter with leucine-H^{3} (2 mC/100 g body weight). Two hours after the isotope was injected, the mice were sacrificed and identical weights of liver from each were pooled. The ribosomes were prepared according to the procedure of Low and Wool (8), modified to include a magnesium chloride

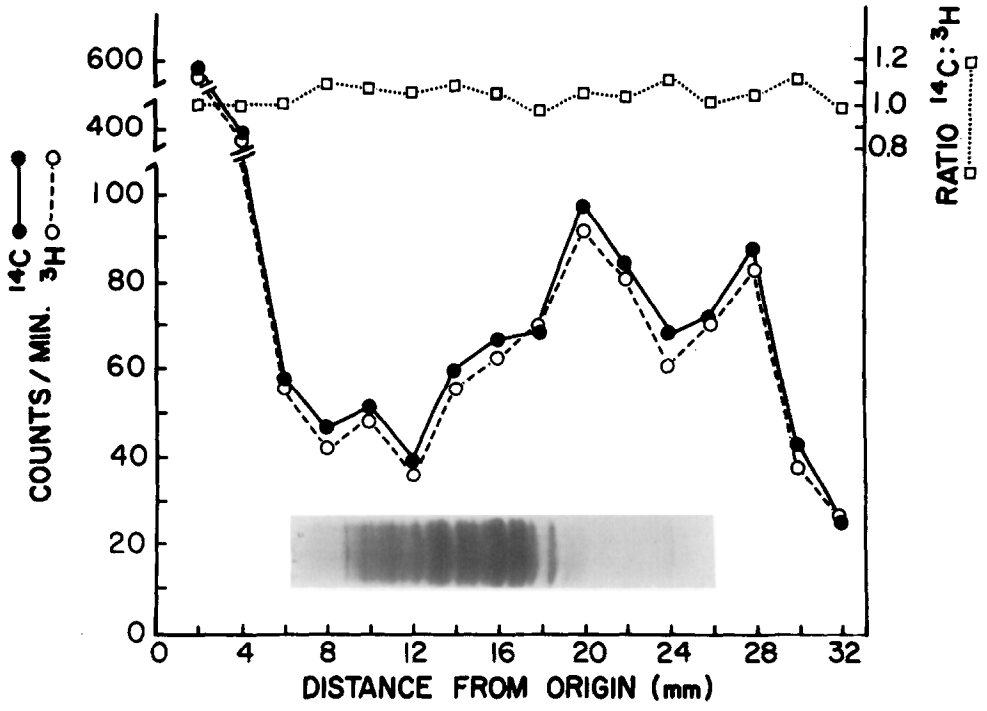

Figure 2: The effect of cortisone on the incorporation of radioactive amino acids into mouse liver ribosomal protein subunits

0.5 ml of the ribosomal protein in 1 mM sodium acetate buffer, pH 4.5, containing 8 M urea was layered over a concentrating gel and a 7.5% separating acrylamide gel column of 0.7 x 7 cm and electrophoresed at pH 4.5 and 4°, at 7 mA per tube for 150 minutes as described by Jovin et al. (5). Following electrophoresis, one gel was stained with Amido Black and the other cut into thirty successive 2 mm slices. Each slice was placed in a scintillation vial, dissolved with 0.1 ml 30% H_2O_2 at 50° C for 1 hour (10) then diluted with 10 ml Bray's solution and its radioactivity measured with a Tri Carb Scintillation counter. After correction for background and quenching, the C^{14}:H^3 ratio was calculated for each slice.

precipitation step to free the ribosomes of ferritin and other extraneous proteins (9). Ribosomal RNA was precipitated by the addition of lithium chloride-urea. After centrifugation, the supernatant containing the ribosomal proteins was dialyzed against 8 M urea to remove the lithium chloride. Ribosomal proteins were fractionated in duplicate by analytical polyacrylamide gel electrophoresis (8). One gel was stained with amido black; 24

distinct bands of ribosomal subunits are clearly resolved with this procedure (Figure 2). The duplicate gel was cut into successive slices which were treated according to the procedure of Tishler and Epstein (10), and their ratio of C^{14}:H^3 determined by liquid scintillation counting with quench correction. It is apparent that the C^{14}:H^3 ratio is constant in each of the ribosomal proteins (Figure 2). Although the structural proteins of the 30S particle have a specific activity higher than those of the 50S,* the hormonal stimulation is the same for every ribosomal protein subunit. The cortisone effect on the ribosomal synthesis is therefore a uniform quantitative acceleration of synthetic rate without alteration in the relative proportions of the structural protein components. These studies also exclude the possibility that cortisone preferentially alters the rate of synthesis of a class of ribosomes of unique subunit composition.

Thus, hepatic enzyme induction by glucocorticoids is not characterized by detectable modification in the rate of synthesis of the structural protein moieties of either the transcriptional or translational cellular apparatus. If these proteins are indeed affected by the hormone, a more subtle alteration of the molecule may occur, involving either changes in acetylation, phosphorylation or free sulfhydryl contents (3, 11).

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