THE EFFECT OF CORTISONE ON THE VOLUME AND TOTAL PROTEIN CONTENT OF MOUSE LIVER NUCLEI

LEONARD AMARAL, LOUIS G. MORIBER, and MARION HIMES. From the Department of Pathology, Methodist Hospital of Brooklyn, 7th Avenue and 6th Street, Brooklyn, N. Y. 11215 and the Department of Biology, Brooklyn College, The City University of New York, Avenue H and Bedford Avenue, Brooklyn, N. Y. 11210

INTRODUCTION

The in vivo administration of cortisone to rats induces the synthesis of specific classes of liver RNA (6, 7) and, consequently, gluconeogenic enzymes (13). Nuclei (4) or isolated chromatin (2) obtained from in vivo coritsone-treated rat liver synthesize RNA in an in vitro system to a greater degree than nuclei or isolated chromatin from untreated controls. Nuclei or chromatin obtained from untreated rat liver, when incubated in a medium containing the hormone, show no increase in their ability to synthesize RNA (4, 2). These results suggest that some component which is present in the in vivo system is absent from the in vitro system. That this component may be a plasma protein is suggested by the facts that: a) cortisone binds selectively to a human plasma protein (8, 11), and b) the perfusion of whole rat liver with medium containing cortisone produces little induction of gluconeogenic enzymes, whereas the addition of whole rat blood to such a perfusion system produces a marked induction (1). It should also be noted that shortly after in vivo administration the hormone is found bound to rat liver histone (12). These findings collectively suggest the following question. Does the hormone enter the liver cell as a hormone-protein complex, and if so, does the mobilized protein remain, thus increasing the total protein content of the nucleus? Until now, biochemical studies have not demonstrated such an increase (4, 2). However, the methods used in these latter studies would permit the loss of soluble proteins (3). In this respect, the use of cytological technics may present a distinct advantage in that the fixation procedure may insure the preservation of most proteins. This paper presents cytological evidence that the administration of cortisone to mice results in an increase in the total protein content of liver parenchyma nuclei.

MATERIALS AND METHODS

Male Swiss albino mice (5 wk old, 20–25 g) were used throughout this study. Control and experimental animals were fed Purina Lab Chow ad libitum and given free access to water at all times. Cortisone acetate (trade name—Cortogen, by Schering Corp., Bloomfield, N. J.) 27 mg/100 g body weight/24 hr, was injected intraperitoneally in the experimental groups. The control animals received an equivalent volume of 0.95% saline. The animals were sacrificed at specific intervals following hormonal treatment, and their livers were removed and sliced at a thickness of less than 2 mm. These slices were processed by the freeze substitution method of Woods and Pollister (14), with methanol as the dehydrating medium followed by hot methanol (60°C) treatment for 1 hr. At the end of the fixation periods, the tissues were embedded in paraffin and sectioned at the appropriate thickness required by the method of analysis employed. All tissues to be compared were mounted on one slide, and thus treated identically.

The determination of the volume of the diploid nucleus was accomplished as follows. Sections were cut at a thickness of 20 μ so that the presence of many whole nuclei would be insured. The slides were stained by the method of Feulgen and mounted in an oil whose refractive index matched that of the cytoplasm (1.556). The identification of a diploid nucleus was obtained by the visual method (5). The volume of each diploid nucleus selected by this method was determined by assuming the nucleus to be a sphere and obtaining the mean of the two principal diameters with the aid of a Cooke image-splitting micrometer. 100 such determinations were made per animal liver and were averaged. The determination of the changes in the total protein per diploid nucleus was performed as follows. Experimental and control livers were sectioned at a thickness of 2 μ, mounted on the same slide, stained with 0.01% fast green, pH 2.00 (pH adjusted with 1 N HCl), and mounted in a refractive index oil of 1.560. Microspectrophotometric extinctions of 3 μ diameter plugs through individual nuclear areas (30 nuclei examined per animal liver) obtained at 590 mμ multiplied by the previously calculated average nuclear volume gives an arbitrary measure of the total amount of stain present per nucleus (9). The ratio of the total amount of stain per nucleus present in the treated liver to that of the control livers provides the second parameter studied.

RESULTS

Table I, summarizes the effect of cortisone on the volume and total protein content of the liver nucleus after 1, 2, 4, 8, 24, and 96 hr of continuous cortisone treatment. It is observed that within 1 hr after initial treatment a barely significant increase in the volume (5%) and total protein content (10%) has taken place. The parallel alterations which are evident at the end of 2 hr, continue to increase almost linearly for at least 8 hr. At the end of 24 hr no further increase in nuclear volume over that found in the 8 hr group is observed, whereas the total protein content has increased 156%. By the end of 96 hr of daily cortisone treatment, both nuclear parameters are near those of the controls.

TABLE I

The Effect of Cortisone on the Volume and Total Protein (Fast Green) Content of Diploid Nuclei

Group	Number of animals	Ave. nuclear volume in μ_3	Number of determinations	±S.E.	Ave. extinction of fast green @ 590 mμ	Number of total nuclear determinations	±S.E.	Ave. amount of fastgreen per nucleus in arbitrary units	Alteration in nuclear volume after cortisone	Alteration in total amount of nuclear fast green after cortisone treatment
									%	%
Control	9	92.2	(900)	±1.19	0.544	(270)	±.029	50.1	—	—
1 hr	3	97.0	(300)	±0.93	0.570	(90)	±.027	55.3	1.05	1.10
2 hr	3	100.4	(300)	±0.96	0.593	(90)	±.029	59.5	1.09	1.28
4 hr	3	107.0	(300)	±0.91	0.669	(90)	±0.041	71.3	1.16	1.42
8 hr	3	132.0	(300)	±1.49	0.780	(90)	±0.033	103.0	1.43	2.56
24 hr	4	132.9	(300)	±1.38	0.605	(90)	±0.035	80.4	1.44	1.60
96 hr	4	94.5	(300)	±1.52	0.550	(90)	±0.028	52.0	1.04	1.02

DISCUSSION

The data presented demonstrate that the in vivo administration of cortisone results in an increase in the volume and total protein content of the mouse diploid nucleus. Evidence has been presented by Shyamala and Gorski (10) that the complex formed between estrogen and a protein enters the uterine nucleus and binds to chromatin. The increase of total nuclear protein observed in our study is probably not a histone increase since an increase in histone unaccompanied by a DNA increase is unlikely. Cortisone has been shown to inhibit DNA synthesis under the conditions used in these experiments (results to be published elsewhere, see Abstract, Amaral, L. 1967, *J. Cell Biol.* **35:** 159A). This study provides circumstantial evidence to support the hypothesis that cortisone binds to a plasma protein (transcortin?) and that this complex enters the liver parenchyma nucleus and consequently binds to chromatin. The inability of cortisone to induce the in vivo effects in an in vitro system which lacks plasma proteins, suggests that the control of specific genetic activity by cortisone is mediated only when such a complex is present. Further investigations may provide further evidence for the existence of such a hormone-plasma-protein complex and may furnish evidence for a mechanism for its action.

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