AN INTERACTION OF CORTISOL WITH COMPONENTS OF LYMPHATIC TISSUE[1]

Willa K. Brunkhorst and E. L. Hess

Worcester Foundation for Experimental Biology

Shrewsbury, Massachusetts

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The hypothesis that hormones function as biocatalysts by reacting with protein structures of the target tissue is attractive. Although considerable information is available concerning the interaction of steroid hormones and plasma proteins, little is known concerning interactions with cellular components. The present study was undertaken to detect interactions of cortisol with components of thymus, an organ which undergoes rapid involution during hormone treatment.

Exploratory experiments utilized a cytoplasmic fraction prepared from frozen calf thymus according to published procedures (Hess and Lagg, 1958) as modified below. Minced tissue was stirred for 10 minutes in 0.0033 M $CaCl_2$. The extract was centrifuged, first at 8500 x g. for 10 minutes, then at 23,000 x g. for one hour. The 23,000 x g. supernatant was dialyzed overnight against water and a ribonucleoprotein fraction precipitated when the pH was lowered to 5.1 with 0.1 N acetic acid. The precipitate (fraction 5.1P) was dissolved in water by adjusting to pH 7 with $NaHCO_3$, dialyzed and lyophylized. RNA content of 5.1P was 11% as determined by the Schmidt-Thannhauser method (1945).

Interactions between fraction 5.1P and cortisol were investigated using the spectrophotometric procedure of Westphal (1957). The absorbance at 248 mμ was determined for 2×10^{-5} M cortisol and a solution of 2×10^{-5} M cortisol containing 0.1% 5.1P. The latter was read against a blank of 0.1%

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solution of the protein. The depression, Δ, of the extinction coefficient of cortisol observed in the presence of 5.1P was calculated according to the relationship.

$$\Delta = \frac{100\ (E_w - E_p)}{E_w}$$

E_w represents the molecular extinction coefficient of the steroid in water and E_p refers to the molecular extinction coefficient of steroid in the presence of protein. Δ was taken as a measure of interaction. Lyophylized fraction 5.1P, dissolved in water at a concentration of 0.1%, usually caused a 20% decrease in absorbance. When the protein was dissolved in 0.0005M $MgCl_2$ or $CaCl_2$ a ΔE as high as 70% was observed. The effect of Mg and Ca ions, while qualitatively consistent, has been quantitatively variable. Random variations in the spectrophotometric readings are due in part, at least, to scattered light resulting from protein aggregation in the presence of these ions.

More reproducible measurements of interaction were obtained using moving boundary electrophoresis. A mixture of cortisol-4-C^{14}, 2-3 μg./ml.* and 5.1P, approximately 12 mg./ml. in 0.05M $NaHCO_3$ was analyzed in a conventional assembly. After passage of 10 ma. for 105 minutes, 0.3 to 0.5 ml. samples were withdrawn from regions in the cell as indicated in Fig. 1. A 22 gauge 18 inch needle attached to a syringe mounted on a rack and pinion was used. Aliquots from these samples were plated and counted in a thin window gas flow counter. Results are listed in Table I.

Samples removed from the descending limb of the cell were essentially free of protein. Steroid present in these regions, therefore, represents the equilibrium concentration of the unbound form. The difference in radioactivity

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Table I

Interaction of cortisol with thymus fraction, 5.1P as determined by moving boundary electrophoresis

Exp.	Initial Solution	Sample D_1	Sample A_1	Sample A_2	Sample A_3	$\frac{\text{Initial} - D_1}{\text{Initial}} \times 100$
1	50,800	36,250	10	238	4090	28.6
2	52,500	42,100	50	335	4640	19.8
3	42,350	33,700	0	185	4490	20.5
4	41,400	32,950	13	528	7520	20.4

Samples were pipetted after electrophoretic transport of fraction 5.1P in 0.05M $NaHCO_3$. See Fig. 1 for regions from which samples were pipetted. Values represent counts per minute per cc. due to cortisol-4-C^{14} present in the samples. Initial solution refers to original solution prior to electrophoretic transport.

of the original solution and the D_1 sample, represents the concentration of cortisol bound to 5.1P.

In the ascending limb of the cell, fraction 5.1P moved into buffer solution which contained no cortisol. Radioactivity found in samples removed from the ascending limb results from transport of cortisol bound to 5.1P. That re-equilibration proceeds rapidly is indicated by the counts from different levels in the cell, A_1, A_2 and A_3.

Control experiments verified that cortisol moved by transport resulting from interaction. Approximately 1% of the counts present in the original solution were found in samples removed from a position immediately above the ε boundary suggesting that diffusion was a negligible factor. In other experiments the total amount of cortisol transported was determined. Ten milliliters of a 1% solution of 5.1P containing cortisol-4-C^{14} was introduced into the cell and allowed to equilibrate between ascending and descending limbs. The bottom section was closed and buffer was layered above the protein. After passage of 36 coulombs the bottom section was closed again. Total volumes in each limb were collected and total cortisol determined. The ascending limb contained 18% more hormone than the descending limb, clearly demonstrating transport of cortisol.

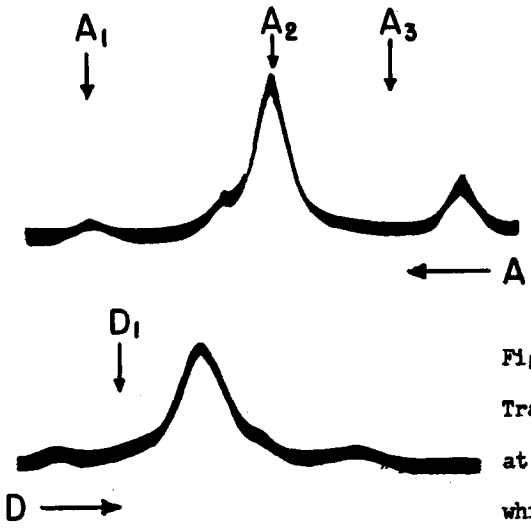

Fig. 1 1.5% 5.1P in 0.05M $NaHCO_3$. Tracing of photograph made after 105 min. at 10 ma. Arrows indicate regions from which samples were pipetted.

Transport experiments were carried out with cortisol added to 0.05M sucrose in $NaHCO_3$. Only 0-2% more cortisol was found in the ascending limb, indicating very little electro-osmosis. In a similar experiment, cortisol was added to 0.3% RNA in 0.05M $NaHCO_3$. The RNA was obtained by extraction of 5.1P with hot 10% NaCl and precipitation with alcohol. In this instance, also, negligible counts were detected in the contents of the ascending limb, suggesting that the RNA moiety of 5.1P did not interact with cortisol. Structural changes resulting from the extraction procedures which may have altered the capacity of RNA to interact with the hormone cannot be excluded, however.

The ultrafiltration method of Toribara (1953) was the most expedient of the methods employed in these studies. Preliminary results using this technique have confirmed the findings of the other methods, i.e., at concentrations used in the experiments 20% of the hormone was bound to 5.1P. RNA prepared from 5.1P gave no indication of interaction, in accord with electrophoretic findings. Failure of free RNA to bind cortisol and the variation in binding obtained when Mg and Ca ions were present suggests possible configurational requirements.

Work reported elsewhere (Hess, Herranen and Lagg, 1961) has demonstrated that fraction 5.1P consists of a mixture of RNA and protein in equilibrium with an RNA-protein complex. The extent of dissociation was found to depend upon pH and ionic strength conditions.

In these studies the fraction of cortisol bound was approximately the same in all cases, even though the ratio of cortisol to protein was varied extensively. One would expect a higher fraction of bound cortisol with low ratios of cortisol to protein. The results obtained suggest that, with varying cortisol concentrations, either the association constant or the number of binding sites changes. The presence in fraction 5.1P of several components capable of binding cortisol provides a third plausible explanation. More extensive studies are in progress investigating the molecular basis of the interaction and its biological significance.

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ERRATA

Volume 4, Number 6, April 28, 1961, in the article entitled "Inhibition of Succinoxidase by L-Gulonolactone Oxidase in Liver Preparations from Tocopherol-deficient Rats" by Paul B. McCay, Abbas E. Kitabchi, Ranwel Caputto and Raul E. Trucco (pages 469-473):

Page 471, line 9 should read "(enzyme plus substrate, expts. 5d, 7b, 8a) to the mitochondrial succin-".

Page 471, line 21 should read "Experiments 6a, b and c show that the activity of this enzyme in the".