THE MAJORITY of living forms depend for their functioning upon two classes of biocatalysts, the enzymes and the hormones. These biocatalysts permit the diverse chemical reactions of the organism to proceed at 38°C with a specificity and at rates frequently unattainable in vitro at elevated temperatures with similar reactants.

The physiologic importance of enzymes and hormones is evident not only under normal circumstances, but is reflected clinically in the diverse descriptions of errors of metabolism, due to lack or deficiency of one or more enzymes, and the numerous hyperand hyperfunctioning states resulting from imbalance of hormonal supply.

Inasmuch as both enzymes and hormones function, with rare exception, to accelerate the rates of processes in cells, investigators have sought possible interrelationships and interactions of enzymes and hormones, particularly as a basis for the mechanism of hormonal action. It has seemed logical to hypothesize that hormones, while not essential for reactions to proceed but nevertheless affecting the rates of reactions, may function by altering either the concentration or activity of the prime cellular catalysts, the enzymes. This proposed influence of hormones on enzymic activity might be a primary, direct effect achieved by the hormone participating as an integral part of an enzyme system, or an indirect influence based upon the hormone altering the concentration of available enzyme and/or substrate utilized by a particular enzyme.

It is the purpose of this presentation to describe a relatively few, but better defined, examples of the more direct relationships of enzymes and hormones. Five examples of enzyme-hormone interaction will be presented, based on the criterion that an effect of the hormone has been demonstrated on addition of the hormone in vitro to a purified, or partially purified, enzyme system. Other additional examples are available in the literature, e.g., the studies describing the influence of deoxycorticosterone and other steroids added in vitro on the activity of a variety of enzyme systems. It is also true that many publications have described alterations in the activity of enzymes in various tissues following administration in vivo of diverse hormonal preparations. However, it is not possible to judge, in the in vivo experiments, whether the reported effects are examples of direct enzyme-hormone interaction, or an indirect influence of the hormone mediated via one or more metabolic pathways, and therefore other enzyme systems whose activities are not being measured. Data from in-vivo studies of this type are thus not pertinent to a discussion of direct hormone-enzyme interaction.

Before considering the main topic of direct enzyme-hormone interaction, it might be well to recall another aspect of enzyme-hormone interaction which is of profound significance for the organism, viz., the alteration of hormonal structure and activity produced by a variety of specific enzyme systems. This information may belong correctly to the subject of hormone metabolism rather than under the topic of possible metabolic influences of hormones at an enzymic level, with which we are concerned in the present discussion. Nevertheless, enzyme-hormone interaction, as seen, for example, in the profound role of the enzymes of the

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Hormone. Enzyme System Affected


<table>
<thead>
<tr>
<th>Hormone</th>
<th>Enzymic System Affected</th>
<th>Effect on Enzymic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenocorticotropin</td>
<td>Adrenal phosphorylase</td>
<td>Increase</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Dephosphophosphorylase phosphokinase (in liver and muscle)</td>
<td>Increase</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Dephosphophosphorylase phosphokinase (in liver)</td>
<td>Increase</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Pyridine nucleotide transhydrogenase (placenta)</td>
<td>Increase</td>
</tr>
<tr>
<td>Adrenal cortical steroids</td>
<td>Adenosine triphosphatase</td>
<td>Increase</td>
</tr>
</tbody>
</table>

Influence of Hormones on Enzymic Reactions

The adenosine 3',5'-monophosphate functions as a cofactor in the enzyme system concerned with the activation of phosphorylase; in the case of liver and muscle, this is the dephosphophosphorylase kinase system. Thus epinephrine and glucagon function to activate phosphorylase, thereby promoting the conversion of glycogen to glucose 1-phosphate, leading to augmentation of the level of glucose in the blood. In the adrenal cortex, it has been hypothesized that an increased activity of adrenal phosphorylase provides additional glucose 6-phosphate,
from which reduced triphosphopyridine nucleotide can be obtained as a consequence of the operation of the phosphogluconate oxidative pathway. In turn, the reduced nucleotide participates in adrenal steroid biogenesis,5-7 notably in the hydroxylation mechanisms which lead to introduction of hydroxyl groups into the steroid nucleus. Haynes and his collaborators8 have recently made the striking observation that the previously named anhydride of adenylic acid functions more efficiently than does adrenocorticotropic in stimulating corticosteroid synthesis in vitro by slices of rat adrenal gland. Thus, although there are marked differences in the end result achieved by adrenocorticotropic, on the one hand, and epinephrine and glucagon, on the other, as a result of their augmentation of the phosphorylase reaction, it is nevertheless of considerable interest that each hormone (a chemically distinct substance from the others) has as a basis for its action at the enzymic level a stimulation of the synthesis of adenosine 3',5'-monophosphate. The mechanism by which these hormones promote this synthesis is at present unknown.

The role of estradiol in augmenting the catalytic activity of an enzyme system present in human placenta, endometrium, mammary gland and the pituitary has been described.9,10 Detailed studies of this system have been conducted with preparations derived from placenta;11,12 it has been demonstrated13,14 that the estrogen-sensitive system catalyzes a pyridine nucleotide transhydrogenation:

\[
\text{TPNH} + \text{DPN}^+ \rightarrow \text{DPNH} + \text{TPN}^+ \cdot
\]

The group of enzymes catalyzing this reaction has been studied extensively15,16 and termed pyridine nucleotide transhydrogenases. It has been proposed14 that the estrogen activates in some unknown manner a specific, soluble placental pyridine nucleotide transhydrogenase. On the other hand, it has been observed17,18 that a variety of 3α-hydroxysteroid dehydrogenases, as well as estradiol-17β-dehydrogenase, could function as transhydrogenases. From this evidence, it was hypothesized that estrogens may function physiologically as coenzymes or cosubstrates in transhydrogenase reactions. For example, by alternately oxidizing and reducing estradiol-17β, the specific dehydrogenase, utilizing different pyridine nucleotides (DPN and TPN) for the two reactions, could effect a net transfer of hydrogen from one nucleotide to another. The hormone thus was visualized as serving as a coenzymic hydrogen carrier. However, it has been reported19 that the mitochondrial transhydrogenases, in contrast to the soluble fractions of human placenta and rat liver, are unaffected in activity by additions of steroid. Moreover, the very low absolute activity of the transhydrogenase reaction catalyzed by hydroxysteroids, and the relatively low activity of the dehydrogenase reaction as compared to the transhydrogenase reaction, make it unlikely that such low activities could be of importance in regulating the flow of electrons between the two forms of the pyridine coenzymes, even in the soluble portion of the cell. It may also be pointed out that it has recently been demonstrated20 that the estrogen-sensitive system of human placenta is a soluble pyridine nucleotide transhydrogenase, separate and distinct from the estradiol dehydrogenase present in the same tissue. It would appear, therefore, that it may be premature to conclude that the physiologic basis for estrogen action, at the enzymic level, has been established.

The fifth and final example of hormone-enzyme interaction to be presented has been studied recently in our laboratory. Certain adrenal cortical steroids, notably deoxycorticosterone and cortisol, have been demonstrated21-24 to produce in vitro a striking inhibition of glucose utilization by cell-free extracts of normal and malignant lymphoid tissue. This effect has been localized in at least two steps in the over-all oxidation of the carbon of glucose to carbon di-
oxide. One of these inhibitory effects of adrenal cortical steroids occurs in the glycolytic pathway, that is, between glucose and lactate, and the other inhibition is evident in the oxidative sequence resulting in the conversion of the carbon of pyruvic acid to carbon dioxide.

Rather detailed study has been made in our laboratory by Dr. Melvin Blecher of the in-vitro inhibition by deoxycorticosterone and by cortisol of anaerobic glycolysis in cell-free preparations of the transplantable Murphy-Sturm lymphosarcoma of the rat. The data reveal that a deficiency in adenosine triphosphate, resulting in part because of an accentuated hydrolysis of the triphosphate by adenosine triphosphatase in the presence of steroid hormone, is one of the causes of the inhibition of glucose utilization produced under these conditions by the added steroid. The inhibition can be overcome, in the presence of steroid, by generating during the incubation period adenosine triphosphate from creatine phosphate and adenosine diphosphate in the presence of purified muscle transphosphorylase. As indicated in Exp. 1a, Table II, in the absence of deoxycorticosterone (DOC) and in the presence of a catalytic quantity of added ATP, the ratio of lactate formed to inorganic phosphate (P_i) taken up is approximately unity and two moles of triose appear per mole of glucose disappearing, as would be predicted on theoretic grounds.

Although all three parameters of glycolysis are decreased in the presence of the steroid (Exp. 1b), there is a disproportionately larger inhibition of the uptake of inorganic phosphate, suggesting an influence of deoxycorticosterone on the release of phosphate to the medium, possibly from ATP. The same relationships obtain when a catalytic quantity of ATP is generated from ADP and phosphocreatine (Exps. 1c and 1d).

When a stoichiometric quantity of ATP is added initially (Exp. 2a), glucose disappearance and lactate formation increase greatly whereas the net inorganic phosphate uptake is augmented only slightly. Our unpublished data indicate that, under these conditions, an increased phosphate uptake into triosephosphates is counterbalanced to a considerable extent by the release of inorganic phosphate from ATP by the action of the tumor ATPase. In the presence of deoxycorticosterone (Exp. 2b) the release of inorganic phosphate into the medium is increased to such an extent that no net uptake of phosphate is observed; concomitantly, glucose disappearance and lactate accumulation are inhibited.

The deoxycorticosterone and ATPase effects can be overcome almost completely when a system is provided which generates a substrate-level quantity of ATP (Exps. 2c and 2d).

The results thus demonstrate an effect of deoxycorticosterone on a cell-free preparation where permeability factors, at least with respect to cell wall membranes, are not involved. The data suggest a role for the steroid in the reactions of anaerobic glycolysis, particularly in those specific reactions involving the utilization and formation of ATP. In this connection, one group reported inhibition of hexokinase in vitro by cortisol, and another described an inhibition of hexokinase in vitro by deoxycorticosterone. However, we have been unable to confirm the data of either of those studies. It may be noted that there has been described an in-vitro combination between ATP and certain steroids, including deoxycorticosterone. A suggested interpretation of our data is that such complex formation between steroid and ATP could be reflected in an influence of the steroid on certain enzymic reactions which are ATP-dependent.

In concluding, it may seem disappointing, in view of the large body of evidence for the participation of certain of the vitamins as coenzymes in well-described enzyme systems, not to have uncovered a similar broad basic principle which would aid in explanation of the influence of hormones on the rates of cellular reactions. However, a significant beginning has been made in dissecting the mode of action of certain hor-
TABLE II
IN-VITRO INHIBITION BY DEOXYCORTICOSTERONE (DOC) OF ANAEROBIC GLUCOLYSIS IN CELL-FREE PREPARATIONS OF RAT LYMPHOSARCOMA

Incubation mixture of 3 ml contains: cell-free preparation from 30 mg tissue, wet wt.; 7.5 µmole potassium phosphate buffer, pH 7.4; 75 µmole KHCO₃; 80 µmole MgCl₂; 46 µmole KCl; 40 µmole KF; 15 µmole potassium pyruvate; 120 µmole nicotinamide; 0.6 µmole K₂DPN; and 10 µmole glucose. Where present, 1.8 µmole DOC. ATP as indicated below. Mixtures incubated for 1 hr at 37.5°C under 95% N₂—5% CO₂ and then deproteinized with 5% trichloroacetic acid. Lactate, Pᵢ, and true glucose determined on trichloroacetic acid filtrates.^{26}

<table>
<thead>
<tr>
<th>Exp.</th>
<th>ATP Source</th>
<th>DOC</th>
<th>(µmole per hour)</th>
<th>∆ Lactate</th>
<th>Pᵢ uptake</th>
<th>∆ Glucose</th>
<th>Pᵢ</th>
</tr>
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<tbody>
<tr>
<td>1a</td>
<td>Present initially*</td>
<td>—</td>
<td>4.35</td>
<td>+8.90</td>
<td>2.20</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>Present initially</td>
<td>+</td>
<td>2.48</td>
<td>+0.90</td>
<td>1.00</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>1c</td>
<td>Generated**</td>
<td>—</td>
<td>4.75</td>
<td>+8.80</td>
<td>3.30</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>Generated**</td>
<td>+</td>
<td>4.25</td>
<td>+1.00</td>
<td>0.70</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>Present initially†</td>
<td>—</td>
<td>7.17</td>
<td>+4.88</td>
<td>7.12</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>Present initially†</td>
<td>+</td>
<td>3.84</td>
<td>—8.18</td>
<td>2.59</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>Generated‡</td>
<td>—</td>
<td>6.68</td>
<td>+0.60</td>
<td>5.60</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>Generated‡</td>
<td>+</td>
<td>5.58</td>
<td>+8.70</td>
<td>4.90</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

* 1.0 µmole K₃HATP (catalytic level).
** 1.0 µmole ATP generated from 1.0 µmole each of K₂HADP and sodium phosphocreatine by 0.3 µg purified transphosphorylase (generously supplied by Dr. Henry A. Lardy).
† 10.0 µmole K₃HATP (substrate level).
‡ 10.0 µmole ATP generated from 10.0 µmole each of K₂HADP and sodium phosphocreatine by 20 µg transphosphorylase.

Monens on enzyme systems. This area of investigation holds promise of rapid expansion in the immediate future. In this regard, a word of caution is perhaps warranted. The precise mechanism of action of hormones, at a cellular level, might not prove to be mediated solely, or perhaps at all, via direct effects on complete enzymic systems. Perhaps more attention should be directed toward an indirect hormonal influence at sites regulating the concentration of components of enzymic systems, that is, the substrates and cofactors made available to the intracellular reactions whose rates are enzymatically controlled.

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