Enzymes in Tissues Responsive to Corticosteroids*

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SUMMARY

The biochemical reactions which mediate the physiological effects exerted by the adrenal cortical hormones remain to be determined. It has been suggested that the hormonal control of cellular functions is accomplished by altering the activity of specific enzymes. This report concerns the induction of several enzymes by cortisol in tissues (liver, thymus, transplantable tumors) which are responsive to the glucocorticoids. Also, some factors involved in studies on the actions of hormones are considered.

The problem of resistance to cortisol and the technics being used to evaluate the physiological significance of enzyme induction by cortisol in target tissues is discussed.

The various dietary and physiological conditions which influence the level of the inducible enzymes are considered in regard to an explanation of the manner in which glucocorticoids alter the activity of the adaptive enzymes.

Several recent articles on the adrenal corticoids have emphasized the disparity between the detailed knowledge available on the chemistry and the biosynthesis or degradation of these hormones and the relative lack of information about biochemical events which underlie their modes of action (15, 25, 46). Although the physiological effects elicited by large doses of the glucocorticoids are well known, it is not yet clear whether such effects are related to the major role these hormones fulfill under normal physiological conditions. These gaps in our knowledge are not limited to the glucocorticoids. The same difficulty has been encountered in elucidating the action at the molecular level of many other hormones (60).

Clinically the glucocorticoids have been shown to be of value in the treatment of leukemias and lymphomas (42). In this regard, the corticosteroids deserve study with respect to the changes they produce in the metabolism of responsive tumors. In keeping with the objectives of this symposium, I do not intend to review in this paper the various mechanisms which have been proposed to explain the action of the adrenal corticoids. Emphasis will be placed almost entirely on the effects of the glucocorticoids on the induction of certain enzymes in those tissues which are targets of the action of the adrenal corticoids. This is a relatively new area of investigation that has revealed interesting facts concerning glucocorticoid action at the biochemical level.

The difficulties in determining the biochemical basis for certain physiological effects exerted by the adrenal cortical hormones are apparent from the many different methods which have been used to study this subject in recent years (7, 41). Most of the studies are predicated on the basis that the hormonal control of cellular functions is mediated by altering the activity of specific enzymes (20, 31). Attractive as this concept may be, it has not yet provided us with a clear-cut relationship between a biochemical event and a physiological effect produced by the glucocorticoids. There has been renewed interest in the possibility that hormones, in general, may act to facilitate the entry of metabolites into cells, mainly because of the recent work on insulin (59) and antidiuretic hormone (1, 9).

Although much of the content of this paper is an account of studies on the action of corticosteroids on intracellular enzymes, there is at present no definitive evidence that this type of effect will turn out to be the basis of their cellular actions. More important at this time is that such studies reveal the need for new information and indicate areas of investigation that deserve attention.

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Some Factors To Be Considered in Studies on the Actions of Hormones

Target Tissues.—Most of the experiments undertaken to gain information concerning metabolic changes which underlie the physiological effects of the glucocorticoids have been carried out in liver. There are only a few reports on changes in lymphoid tissues and certain neoplasms that are remarkably responsive to these steroids. However, there are many studies on the metabolism of glucocorticoids by target cells (11). Tissues of this type which show such a rapid and demonstrable response to corticosteroid therapy are capable of yielding important biochemical information related to the effects exerted by these hormones. A major objective of our work is to determine the basis for the selective action of glucocorticoids on lymphoid neoplasms. The manner whereby adrenal corticoids impair the growth of lymphoid tumors may bear certain similarities to the processes underlying thymic involution or the catabolism of protein.

In vitro vs. in vivo studies.—The choice between in vitro and in vivo experiments is often determined by the problem under investigation and by the techniques familiar to the investigator. Engel (15), Villee (63), and Bush (7) have recently emphasized the importance of the in vitro experiment to obtain unequivocal evidence for a hormonal effect. This approach is often justified on the basis that it permits adequate control of many of the variables that make it difficult to interpret studies in the whole animal. However, it is not yet clear whether the physiological or pharmacological effects of a hormone are entirely mediated by a direct effect of the hormone on responsive tissues.

In view of our limited knowledge about hormone action, it would seem that the experiment in which the test substance is administered to the animal and the tissues subsequently examined for certain biochemical changes be given preference at this time over the experiments involving addition of the hormone to an in vitro system. The in vivo experiment is likely to provide us initially with the important leads concerning metabolic changes related to glucocorticoid action. There appear to be few examples of any substantial effect of glucocorticoids in low concentration on slices or homogenates of liver. The findings of Chiu and Needham (8) that adrenal cortical extract increased liver glycogen of surviving liver slices in vitro have only recently been confirmed (24). The induction of certain enzymes that can be readily shown to occur in the liver of rats treated with glucocorticoids has not been clearly demonstrated in liver slices (19) but does occur in liver perfused with cortisol (3, 19).

Amount of hormone and its specificity of action.—Bush (7) has recently commented in detail on one of the difficulties encountered in studies in vitro—namely, the choice of a reasonable concentration of adrenal hormone. Frequently, in such studies, amounts of the steroid far in excess of a reasonable physiological concentration are required to produce a significant effect. For the same reason, the high dosage used in many in vivo studies has been subject to criticism. Probably undue criticism has been given to the use of large amounts of a hormone in these different systems. Many examples can be cited in which the use of “unphysiological” amounts of a compound has contributed significantly to an understanding of its mechanism of action and pathways of metabolism.

Other criteria must also be kept in mind when assessing hormone experiments. The interpretation of the data is difficult when the effect produced is not specific for a given class of hormones or when an effect is obtained with an analog that is inactive in the animal. Numerous studies can be cited in which a variety of hormones with different structures and physiological properties act similarly in their effect on a biochemical reaction (5, 14, 23, 68). Also, to be of significance in an in vitro system, the effects observed should bear some discernible relationship to the effects of the hormone in vivo, with allowances being made for differences in metabolism in the two systems.

Enzymes Responsive to Glucocorticoids

It is probable that alterations in the activity of certain enzymes underlie the pronounced effects of glucocorticoids on carbohydrate and protein metabolism. In this regard, it is of interest that most enzymes which show an increase in activity after glucocorticoid treatment are involved in the metabolism of glucose or amino acids (52).

Enzyme studies in different tissues of adrenalectomized animals have been carried out in an effort to reveal metabolic functions which are influenced, at least in part, by adrenal hormones (50). A drop in enzyme activity following adrenalectomy has in many instances provided circumstantial evidence that the enzyme is under the control of the adrenal hormones. Whereas many of the adaptive enzymes involved in amino acid metabolism show a decrease in level after adrenalectomy, a number of the carbohydrate-metabolizing enzymes that respond to glucocorticoids do not undergo any significant change in activity (85). The factor(s) which maintain normal hepatic...
levels of certain glucocorticoid-inducible enzymes in the liver of adrenalectomized animals are not known.

Carbohydrate-metabolizing enzymes.—The administration of cortisol to rats for 5–7 days increased the hepatic activity of glucose-6-phosphatase to values 50–100 per cent above normal (64). The activity of this enzyme is increased under conditions which stimulate gluconeogenesis, suggesting that it may have a role in the production of glucose in liver. Evidence for the hormonal and physiological regulation of the level of glucose-6-phosphatase in liver has been thoroughly discussed in a review by Ashmore and Weber (2). Fructose-1,6-diphosphatase also responds to cortisone in intact (37) and adrenalectomized rats (52, 65), whereas other enzymes involved in glycolysis (phosphohexose isomerase, phosphoglucomutase, lactic dehydrogenase) were found to be stimulated by cortisol in the liver of adrenalectomized rats (65).

Amino acid-metabolizing enzymes.—Various enzymes that function in the metabolism of amino acids have been shown to be responsive to the glucocorticoids. These include tryptophan pyrrolase, tyrosine-, alanine-, and tryptophan-a-ketoglutarate transaminase, and the urea cycle enzymes (592). Significant increases in the activity of each of these enzymes have been demonstrated in the liver of rats treated with cortisone or cortisol. Other transaminases such as aspartic-a-ketoglutarate and histidine- and phenylalanine-pyruvate do not respond to cortisol. No answer is yet available as to why some transaminases respond to cortisol and others do not. However, there is no reason to believe that the list of enzymes which act within this area of metabolism and which respond to cortisol is complete.

Tryptophan pyrrolase has been studied extensively by Knox and co-workers (30) and by others (18, 61). This enzyme can be induced in the liver of the intact rat by the injection of many different types of compounds (28). Indoles, including tryptophan, and glucocorticoids are the most potent inducing agents and are the only compounds effective in stimulating the activity of this enzyme in adrenalectomized rats (50). The response of tryptophan pyrrolase to cortisol in rat liver was dose-dependent, and it was suggested that this method could serve as a bioassay for glucocorticoids (69).

A marked increase in tyrosine transaminase of rat liver after administration of tyrosine or cortisol was first observed by Lin and Knox in 1957 (55). Although several aspects of the induced response of this enzyme were similar to those previously noted for tryptophan pyrrolase, there were also certain notable differences. Adrenalectomy alone did not lower the level of this enzyme in liver, and when tyrosine was administered to adrenalectomized rats, the activity of the enzyme was not increased. Cortisol proved to be an effective inducer of tyrosine transaminase in both intact and adrenalectomized rats. More recent studies by Kenney and Flora (57) have indicated that tyrosine is not specific as an inducer of this transaminase. The administration of tryptophan, 6-L-5-OH-tryptophan, or serotonin has been found to produce significant increases in the level of tyrosine transaminase in the liver of intact and adrenalectomized rats (50). Thus, these indoles would appear to be acting in some specific manner, and not via a stress-mediated adrenal response, to induce the activity of this enzyme.

It has long been considered an attractive possibility that cortisone could increase gluconeogenesis and impose a negative nitrogen balance by enhancing transamination reactions (38). In an earlier study, Beaton and co-workers (4) found that daily treatment of adult rats with cortisone did not affect aspartic transaminase in liver but doubled the activity of alanine transaminase. In 1957 Gavosto et al. (17) reported that almost toxic doses of cortisone (130 mg/kg) administered to rats daily for 8 days resulted in a 57 per cent increase in aspartic transaminase activity in liver and an 81 per cent rise in the level of alanine transaminase. Studies in our laboratory revealed as much as a 500 per cent increase in alanine transaminase activity in livers of rats treated with cortisol for 7 days, whereas, under the same conditions, the values for aspartic transaminase were not significantly different from those in the untreated control animals (63). Only those steroids which are active as glycogenic and anti-inflammatory agents induce an increase in the activity of alanine transaminase in liver (54). A direct relationship between the alanine transaminase response and the dose of cortisol administered was observed (55). The initial response to treatment with glucocorticoids involves a rapid increase in the availability of glucose which can result in measurable glycogen deposition within several hours. The slow response, over a period of several days, of alanine transaminase in the liver of rats treated with cortisol correlates with the response to prolonged stress rather than with the initiation of gluconeogenesis. It would seem, however, that this transaminase is capable of regulating the capacity of the liver for gluconeogenesis (59).

Treatment with deoxycorticosterone lowered the level of alanine transaminase in rat liver to 50
per cent of the normal values. Since deoxycortico-
stosterone was ineffective in this regard in hy-
ophysectomized or adrenalectomized rats, its
inhibitory effect on this enzyme in intact animals
has been attributed to interference with the release
of corticotrophin from the pituitary (21).

In other studies it was found that treatment of
rats with testosterone, progesterone, growth hor-
mone, or insulin did not stimulate an increase in
hepatic levels of alanine transaminase, whereas
estrogen and thyroxine caused a slight rise in the
activity of this transaminase (54).

**Table 1.** Recently, detailed information on this
enzyme has been reported (40), and the effects of
various physiological conditions on the activity of
many enzymes have been reviewed (59).

The conditions listed in Table 1 (fasting, high
protein intake, and alloxan diabetes) known to re-
sult in an enhanced rate of gluconeogenesis cause
marked increases in the level of hepatic alanine
transaminase and have also been found to cause
increases of about the same magnitude in the
activity of tyrosine transaminase and tryptophan
pyrrolase (20). However, in many of these condi-
tions, marked differences in the response of several
of the cortisol-responsive enzymes have been ob-
served. For example, although alanine transami-
nase and tryptophan pyrrolase are significantly
lowered in activity following adrenalectomy, the
level of tyrosine transaminase in liver is unchanged
after removal of the adrenals. Also, although alanine
transaminase and to a lesser extent trypto-
phane pyrrolase show a rise in specific activity in
liver with age, tyrosine (58) and aspartate trans-
aminase (22) levels are not affected by aging.

Hypophysectomy, which raises the activity of
alanine transaminase, is without significant effect
on the hepatic levels of tryptophan pyrrolase and
tyrosine transaminase.

In confirmation of the earlier observation by
Curry and Beaton (10), the level of alanine trans-
aminase in the liver of pregnant rats is decreased,
and it cannot be induced by doses of cortisol as
large as 100 mg/kg injected during the last several
days of pregnancy. In contrast, pregnancy did not
depress the activities of tyrosine transaminase or
tryptophan pyrrolase in rat liver or impair the in-
duction of these enzymes by cortisol. Although
several explanations have been proposed to ac-
count for the failure of hepatic alanine transami-
nase in pregnant rats to respond to cortisol (10,
59), none of these can satisfactorily explain why
two other inducible enzymes are not similarly
affected.

It is of interest that the activity of alanine
transaminase can be increased in intact or adenal-
lectomized rats by feeding diets containing a high
content of protein, whereas the substrates of this
enzyme when injected or fed in the diet do not
stimulate an increase in its activity. When cortisol
was administered to adrenalectomized rats main-
tained on different levels of dietary protein, a sig-
ificant increase in the activity of alanine transami-
nase was observed. These findings suggest that
the availability of substrates, although impor-
tant, is not the only factor affecting the induc-
bility of this enzyme by cortisol.

**Effects of Certain Physiological
Conditions on Inducible
Enzymes**

Glucocorticoids are thought to play a dominant
role in the regulation of enzyme activity, but there
is much evidence that dietary modifications and
certain altered physiological states often can exert
parallel and equally pronounced effects on enzyme
systems. These findings provide insight concern-

ing possible mechanisms of corticosteroid action
and suggest new experimental approaches to this
problem. Conditions which alter the level of al-
anine transaminase in liver, or change the in-
ducibility of this enzyme by cortisol, are given in

**Table 1.**

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Response*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol-treated</td>
<td>5- to 7-fold increase in 1 week</td>
<td>(42)</td>
</tr>
<tr>
<td>Aging</td>
<td>6-fold increase in 1 year</td>
<td>(50)</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>60% decrease in mature animals only</td>
<td>(50)</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>5 to 8-fold increase</td>
<td>(44)</td>
</tr>
<tr>
<td>Fasting</td>
<td>5-fold increase after 4 days</td>
<td>(44)</td>
</tr>
<tr>
<td>50% Protein diet</td>
<td>Increase after one week, 4-fold (intact), 2.5-fold (adrenalectomized)</td>
<td>(44)</td>
</tr>
<tr>
<td>75% Protein diet</td>
<td>Increase after one week, 7.5-fold (intact), 4-fold (adrenalectomized)</td>
<td>(44)</td>
</tr>
<tr>
<td>Alloxan-diabetes</td>
<td>3- to 5-fold increase within 1 week</td>
<td>(44)</td>
</tr>
<tr>
<td>X-ray treated</td>
<td>50% increase within 2 days</td>
<td>(51)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>50% decrease between days 15 to 21</td>
<td>(53)</td>
</tr>
<tr>
<td>Pregnancy + cortisol†</td>
<td>No significant increase</td>
<td>(82, 58)</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>70% decrease within 48 hours</td>
<td>(53)</td>
</tr>
<tr>
<td>Fetal animals</td>
<td>Values 50% lower than in newborn</td>
<td>(50)</td>
</tr>
</tbody>
</table>

* In reference to the level in liver of unoperated control rats about 6 weeks of age fed a stock diet.
† 20-100 mg of cortisol/kg daily administered for several days after the 10th day of pregnancy.
significant but less than maximum increment in alanine transaminase activity was observed with the 0 and 25 per cent protein diets, whereas the 50 and 75 per cent protein diets permitted a maximal response to cortisol. Thus, the maximal alanine transaminase response in the liver of adrenalectomized rats appears to be attributable to an additive effect of both dietary protein and administered cortisol.

**ENZYME INDUCTION IN TARGET TISSUES AND IN EXPERIMENTAL TUMORS**

It was of considerable interest to find that the activity of alanine transaminase could be markedly increased in tissues other than liver which are responsive to treatment with the glucocorticoids (54). Comparative data for induced enzyme activity in liver, thymus gland, and the cortisol-sensitive tumor Walker carcinoma 256 are shown in Chart 1. A thirteen-fold increase in alanine transaminase activity was observed in thymus tissue following daily injections of cortisol for 1 week. This treatment resulted in a 95 per cent reduction in the size of the thymus gland. Similarly, treatment with cortisol inhibited the growth of the Walker tumor by about 90 per cent and increased the activity of alanine transaminase in this tissue fourteen-fold. In the same tissues, the activity of aspartate transaminase was only slightly increased, indicating that the rise in alanine transaminase activity is not due to concentration of this enzyme in residual tissue. In other studies, the level of tyrosine transaminase in thymus and Walker tumor was unaffected by treatment with cortisol.1

**TABLE 1**

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>CORTISOL</th>
<th>RELATIVE TRANSAMINASE ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>THYMUS*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>WALKER**</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CARCINOSARCOMA 256</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* 2.5 mg. cortisol s.c. per rat per day for 7 days
** 3.0 mg. cortisol s.c. per rat per day for 7 days

**CHART 1.** Specificity of transaminase response in tissues sensitive to corticosteroids

**Response of Walker carcinoma 256 to corticosteroids.**—The growth of the Walker tumor was suppressed, and the activity of alanine transaminase was increased significantly in animals receiving cortisol (10–50 mg/kg daily for 14 days). The effect of cortisol on this tumor was not related to reduced dietary intake and loss of body weight. When tumor-bearing rats were treated with 30 mg deoxycorticosterone/kg for 2 weeks, the transaminase activity in the tumor was reduced by 50 per cent and, concomitantly, the rate of growth of the tumor was increased consistently (Table 2). Thus, an inverse relationship between the activity of alanine transaminase and the growth of the tumor was observed.

**TABLE 2**

<table>
<thead>
<tr>
<th>TREATMENT*</th>
<th>TUMOR GROWTH</th>
<th>ALANINE TRANSAMINASE ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>(mg/kg/day)</td>
<td>(Per cent control) (mMoles substrate utilised/gm protein/hr at 58° C.) Treated/control</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>0.92 ± 0.06†</td>
</tr>
<tr>
<td>Cortisol</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Deoxycorticosterone acetate</td>
<td>30</td>
<td>148 (P &gt; 0.05)</td>
</tr>
</tbody>
</table>

* Compounds were injected subcutaneously for 14 days; treatment began 24 hours after the tumor was transplanted.
† The animals were sacrificed and the tumors weighed 15 days after transplantation.
‡ Average value ± standard deviation.
Walker tumor could be demonstrated with respect to both inhibition and stimulation of tumor growth in rats treated with cortisol or deoxycorticosterone (49).

Another aspect of this study was concerned with the growth and transaminase activity of the tumor in animals treated with cortisol by three different routes of administration (49). Tumor growth was most effectively suppressed when the steroid was given subcutaneously. Maximum increases in the alanine transaminase activity of liver and tumor were also associated with this mode of administration. The inhibition of tumor growth (about 50 per cent) observed in animals given cortisol orally or intraperitoneally was not associated with a change in tumor alanine transaminase activity, although the level of this enzyme in liver was elevated. Slow absorption of cortisol from a subcutaneous depot appears to be necessary for maximal inhibition of the growth of the Walker tumor and for the greatest increase in liver and tumor alanine transaminase activity.

The activity of alanine transaminase was also examined in the Walker tumor of animals fed high protein diets, fasted, or made diabetic by alloxan (49). Each of these conditions was previously shown to stimulate the activity of this enzyme in liver to an extent comparable to that obtained by injection of cortisol. In comparison to rats fed a purified diet containing 18 per cent casein, the growth of the tumors in the animals maintained on the 50, 75, and 95 per cent protein diets was reduced significantly. Maximum inhibition of tumor growth was obtained in the rats fed the 75 per cent protein diet. Associated with reduced tumor growth, there was a three-fold increase in the alanine transaminase activity in the tumors of the rats fed the 75 and 95 per cent protein diets.

The several-fold increase in tumor alanine transaminase activity observed in animals fed high protein diets or in rats injected with cortisol was not observed in the diabetic or fasted animals, even though marked inhibition of tumor growth was observed in each case. Suppression of tumor growth in diabetic or fasted animals may result from impairment of a number of different metabolic functions, each important for growth. The increase in alanine transaminase activity in both liver and tumor in rats treated with cortisol or fed a high protein diet might be associated with higher levels of circulating amino acids than would occur in animals in which the liver is converting amino acids to carbohydrates that are required as a source of energy by the diabetic or fasted animal.

Comparison of glucocorticoid-sensitive and -resistant lines of a lymphosarcoma.—The development of cortisone-sensitive and -resistant lines of lymphosarcoma P1798 was described in detail by Lampkin and Potter (38). When F1 mice (BALB/c X DBA/2) bearing established P1798 tumors are given subcutaneous injections of 1 mg. of cortisol daily for 4 days, the sensitive line undergoes almost complete regression, but the growth of the resistant tumor is not impaired. It was of interest to determine whether the sensitive line of this tumor would respond in the same way as the Walker tumor with respect to stimulation of alanine transaminase activity and whether the refractory tumor would not show a change in the activity of this enzyme (51). The effects of cortisol on growth and alanine transaminase activity of the cortisolsensitive and -resistant lines of lymphosarcoma P1798 are shown in Table 3. In animals treated with 50 mg cortisol/kg daily for 4 days, the growth of the cortisol-sensitive tumor was reduced to one-fifth the weight of the control tumors, and transaminase activity was increased three-fold. A significantly greater reduction in tumor size and a nine-fold increase in alanine transaminase activity resulted when 100 mg cortisol/kg was administered. The growth of the cortisol-resistant tumor was not affected by treatment with the smaller dose of cortisol, whereas the 100 mg/kg dose reduced tumor growth by 50 per cent. In each case, the changes observed in alanine transaminase activity were not considered significant.

Table 4 shows the effects of cortisol treatment on the tyrosine- and aspartic-a-ketoglutarate transaminase activity of the cortisol-sensitive and -resistant lines of P1798. The tyrosine transaminase activity of the sensitive tumor was increased several-fold by treatment with cortisol. In the resistant tumors, the administration of cortisol resulted in a 60 per cent rise in the activity of this transaminase.

In previous studies, aspartate transaminase was found to be refractory to treatment with cortisol in liver, thymus gland, and Walker tumor. However, in the cortisol-sensitive lymphosarcoma, the activity of this transaminase was observed to be significantly lower than the control value following treatment of the animals with cortisol. The significance of this finding is not clear but is of interest insofar as it provides a different example of a change in transaminase activity induced by a glucocorticoid.

In contrast to the action of deoxycorticosterone in stimulating growth and lowering the alanine transaminase level in the Walker tumor, neither the growth nor the activity of alanine or
aspartate transaminase in the cortisol-sensitive and -resistant lines of P1798 was affected by treatment with this mineralocorticoid.

Altered alanine transaminase activity related to the sensitivity of tumors to cortisol.—Our work to date has indicated a possible relationship between changes in the levels of alanine transaminase and the responsiveness of several tumors to treatment with cortisol (Chart 2). Additional results on other tumors are needed before this relationship is clearly established.

Mode of Action of Glucocorticoids
It is the purpose of studies on the mechanism of action of the glucocorticoids to determine the rate of interaction of the steroid with the receptor in

### TABLE 3

<table>
<thead>
<tr>
<th>TREATMENT*</th>
<th>NO. MICE</th>
<th>TUMOR GROWTH</th>
<th>ALANINE TRANSAMINASE ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(GRAMS)</td>
<td>(PER CENT CONTROL)</td>
</tr>
<tr>
<td>CORTISOL-SENSITIVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7</td>
<td>2.9</td>
<td>0.31</td>
</tr>
<tr>
<td>50 mg cortisol/kg</td>
<td>4</td>
<td>0.6</td>
<td>21.95</td>
</tr>
<tr>
<td>100 mg cortisol/kg</td>
<td>4</td>
<td>0.28</td>
<td>8</td>
</tr>
</tbody>
</table>

CORTISOL-RESISTANT

|          |          |               |                               |                               |
| None     | 9        | 3.4           | 0.28                          |                               |
| 50 mg cortisol/kg | 2       | 3.4           | 100                           | 0.50 |
| 100 mg cortisol/kg | 4      | 1.8           | 52                            | 1.5  |

* The amount of cortisol indicated was injected subcutaneously daily for a period of 4 days.

### TABLE 4

<table>
<thead>
<tr>
<th>TREATMENT*</th>
<th>NO. MICE</th>
<th>TRANSMNASE LEVELS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TYROSINE</td>
<td>ASPARTATE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACTIVITY</td>
<td>TREATED/CONTROL</td>
</tr>
<tr>
<td>CORTISOL-SENSITIVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>0.023</td>
<td>21</td>
</tr>
<tr>
<td>50 mg cortisol/kg</td>
<td>8</td>
<td>0.061</td>
<td>2.7</td>
</tr>
<tr>
<td>100 mg cortisol/kg</td>
<td>4</td>
<td>0.14</td>
<td>14</td>
</tr>
</tbody>
</table>

CORTISOL-RESISTANT

|          |          | 0.023 | 25               | 1.1 |
|          |          | 0.036 | 27               | 1.1 |

* The amount of cortisol indicated was injected subcutaneously daily for a period of 4 days.

† µMOLES SUBSTRATE UTILIZED/GM PROTEIN/HR AT 38° C.
the target cell and the subsequent metabolic
changes that account for the physiological and
morphological effects noted in the responsive tis-
ture. Although there is a large body of information
on this subject, the actions of this class of hor-
mones at the molecular level remain unexplained.
There is now considerable evidence that one of the
biochemical effects elicited by the glucocorticoids
involves the synthesis of enzymes. Therefore, it
seems appropriate in this concluding section to
consider the manner in which glucocorticoids
might influence the level of certain intracellular
enzymes. Attention will also be given to the prob-
lem of resistance and the technics we are using in

an attempt to evaluate the physiological sig-
nificance of enzyme induction by cortisol in target
tissues.

The major physiological effects produced by the
glucocorticoids include an accelerated rate of
 gluconeogenesis, the synthesis and depositions of liver glycogen, enhanced protein catabolism, and a cytolytic action on lymphoid tissue. The hor-
monal specificity of the alanine transaminase re-
sponse to cortisol, the magnitude of this effect, as
well as the gluconeogenic potency of the sub-
strates (pyruvate, alanine, glutamate) of this
transaminase suggested that the cortisol-regulated
hepatic levels of this enzyme could serve as part of
a mechanism which regulates the capacity for
gluconeogenesis (55). Added support for this hy-
pothesis was obtained when it was found that the
levels of this enzyme in liver could be readily in-
creased in three other conditions which are known
to stimulate gluconeogenesis, such as high protein
intake, diabetes, and fasting (55).

Other evidence has been presented that this
enzyme might be rate-limiting for glycogen syn-
thesis initiated by cortisol treatment. In the pyri-
doxine-deficient animal, pyridoxal phosphate, the
co-factor of alanine transaminase, would be ex-
pected to be limiting, and thus the response of this
enzyme to cortisol would be impaired. Utilizing
this technic, Eisenstein (13) demonstrated a dimin-
ished glycogenic and alanine transaminase
response to cortisol in pyridoxine-deficient rats.
These results have been confirmed in somewhat
similar studies in our laboratory (48). The increase
in activity of alanine transaminase after treatment
with cortisol was almost totally blocked in liver
and inhibited markedly in the thymus of rats de-
pleted of pyridoxine. However, whereas the failure
of the glycogenic response paralleled the impaired
enzyme response in liver, cortisol exerted its usual
thymolytic action despite the reduced response of
the transaminase observed in this tissue. These
data do not unequivocally demonstrate a correla-
tion between glycogen synthesis in liver and levels
of alanine transaminase activity as influenced by
cortisol. Since pyridoxal phosphate is a co-factor
for many enzymes, the possibility exists that pyri-
doxine deficiency may affect some other system
that is involved in glycogen synthesis.

This technic was also used in studies concerned
with a possible correlation between the growth of cortisol-sensitive tumors and their alanine
transaminase response to cortisol. In experiments
to date, the inhibition of growth of the Walker
tumor and of lymphosarcoma P1798 in pyridoxine-
deficient animals treated with cortisol was equal
to that obtained in animals bearing these tumors but receiving a complete diet. However, since the alanine transaminase response to cortisol in the
tumors was not significantly impaired by the B6
deficiency, no definite conclusions can be drawn
from these findings. Until the stimulation of this
enzyme by cortisol can be completely inhibited, as
it is in the liver of the B6-deficient rat, it will not be
possible to clearly interpret the data.

Another approach to this problem involves the
combined use of a compound which inhibits pro-
tein synthesis, such as puromycin or ethionine,
with cortisol in an attempt to block the effects of
the steroid on tumor growth. Mueller (38) has
presented evidence that many of the biochemical
effects that occur in the uterus subsequent to
estrogen treatment are mediated by the synthesis
of new protein. It would also appear that the syn-
thesis of enzyme protein is responsible for most of
the increase in the activity of several enzymes in-
ducible by glucocorticoids (16, 26, 57). Ethionine or puromycin have effectively prevented the induction in liver of alanine (56) and tyrosine transaminase (35) and tryptophan pyrrolase (45) by cortisol. In one preliminary experiment, puromycin injected at 2-hour intervals over a period of 6 hours, starting 1 hour before the administration of cortisol, failed to prevent the inhibition of growth of lymphosarcoma P1798 and also did not abolish the response of alanine or tyrosine transaminase in the tumor. Additional studies of this type are needed to reveal whether the synthesis of enzymes in lymphoid tissues following cortisol therapy is of significance with respect to the action of the glucocorticoids on these tissues.

Differences have been noted in the way in which target cells metabolize corticosteroids that are not only related to cell type but also to the stage of differentiation and function of particular cells (11, 12). The basis for the response of lymphocytes appears to be related to the presence of 11-β-hydroxysteroid dehydrogenase, an enzyme which catalyzes the interconversion of cortisone and cortisol. Factors which shift the equilibrium of this enzyme toward the formation of cortisone tend to favor lymphocytopenesis, whereas conditions which permit the accumulation of cortisol favor a lymphocytic action. The two lines of lymphosarcoma P1798 would be ideal tissues in which to study the possible relationship of such an enzyme, and the reaction it mediates, to the sensitivity or resistance of a malignant tissue to cortisol.

What other factors might account for the resistance of a lymphoid neoplasm to adrenal corticoids? The lack of responsiveness of the malignant tissue may simply be due to the failure of cortisol to penetrate the cell. Thus, a change in the structure of the cell membrane or of a system which regulates the transport of the steroid could conceivably alter sensitivity to the corticosteroids. Wilmer (66) has suggested that the properties of the cell membrane could be altered by steroids combining with the phospholipide-cholesterol layer. Since the resistant form of P1798 was developed by exposure of the sensitive line to cortisol during numerous transplant generations, it might have a modified membrane that impedes the entry of glucocorticoids into the cell.

As compared with the significant changes in the activities of alanine, tyrosine and aspartate transaminase observed in the sensitive line of P1798, the resistant tumor showed only slight alterations in the levels of these enzymes. It is possible that the protein-forming systems which control the level of the adaptive enzymes, alanine and tyrosine transaminase, are impaired in the resistant cell line. A relevant question, the consideration of which is outside the scope of this paper, concerns the possibility that in certain malignant cells there is a defect in the capacity for regulation of the level of enzymes which are normally rate-limiting in the control of metabolism. This hypothesis has been proposed by Potter (44) and Pitot (43) and will be considered elsewhere in this conference.

In microorganisms, the synthesis of specific enzymes involves complex control processes. Substrates or closely related compounds can induce the synthesis of many enzymes. The counterpart of induction is repression, by which specific substrates repress the synthesis of particular enzymes. It is not known whether enzyme repression is a general phenomenon in higher animals. If this should prove to be the case, is it possible that glucocorticoids which stimulate enzyme induction act in this respect by interfering with the formation or availability of particular repressors?

A hypothetical mechanism by which glucocorticoids stimulate enzyme synthesis must take into account the various dietary and physiological conditions which increase the activity of the inducible enzymes, as well as those conditions which depress the level of these enzymes (Table 1). An attempt has been made to include all these observations, as they apply to alanine transaminase in liver, into a tentative explanation (Chart 3) of enzyme induction.

The treatments or conditions which lead to an increase in activity of alanine transaminase have been associated with an increase in the concentration of amino acids or protein precursors in liver. On the other hand, pregnancy, tumor growth, and heptectomy involve the growth of new tissues, which requires amino acids for protein synthesis. Thus, under these conditions, the free amino acid pool in liver would be expected to be decreased.

Treatment with cortisol has been found to impair the mobilization of albumin and globulin in
liver (47) and the synthesis of muscle protein (67). Furthermore, cortisol has been shown to facilitate the uptake of amino acids into liver (41).

It is conceivable that competition for amino acids may limit the capacity of the specific ribosomal RNA which functions as a template for the synthesis of alanine transaminase to operate at maximum efficiency. If this were the case, then those conditions which enlarge the amino acid pool would be expected to favor the induction of such an enzyme, whereas conditions which lower the concentration of protein precursors would tend to depress its level in liver.

However, although the increased availability of protein precursors may be a prerequisite for increased enzyme synthesis, this still does not explain specific synthesis and degradation of proteins, often in the same tissue.

In conclusion, it would seem that using adaptive enzymes as model systems offers certain advantages for studies of (a) factors that are involved in the control of metabolism, (b) the manner whereby glucocorticoids can influence enzyme synthesis, and (c) the significance of changes in enzyme activity with respect to the physiological effects produced by glucocorticoids in target tissues.

Further progress in this area awaits the answers to several questions. What is the primary site of action of the glucocorticoids which results in an increase in enzyme activity? What determines the inducibility of only certain enzymes by the glucocorticoids? Use of radioisotope-labeled hormones and amino acids, and the availability of techniques for cell fractionation and the study of protein synthesis in vitro, should soon make it possible to obtain answers to these and other questions concerning hormone-enzyme relationships.

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