THE METABOLIC EFFECTS OF CORTISONE

I. THE OXIDATION OF PROLINE

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With the greatly increased medical interest in cortisone (17-hydroxy-11-dehydrocorticosterone, Kendall's Compound E, Cortone¹) and the development in the past few years of methods for maintaining activity in homogenates of tissues, it appeared desirable to determine the effects of adrenalectomy or treatment with cortisone demonstrable with reactions measurable in tissue homogenates. A considerable portion of the work previously done on tissue metabolism has been reviewed elsewhere (1-4), and it is apparent from even a cursory examination of the literature that two conditions predominate. First, a variety of metabolic effects is observed, rather than a single more or less specific alteration in metabolism. Second, the reports from different laboratories show rather marked discrepancies as to the metabolic effect of adrenalectomy and treatment with cortical extract. These conditions give rise to a state of uncertainty as to just what the metabolic results of cortisone are and suggest that a multiplicity of effects is being studied simultaneously and attributed to a single cause.

Previous studies on the metabolism of tissues of adrenalectomized animals have left two impressions: first, that there is a decreased ability of the tissue to oxidize a variety of substrates and, second, that there is a decreased power of deamination. These impressions have been derived from essential data in the literature (5-17). Summaries and discussion of the significance of these observations are given by Hartman and Brownell (1) and Pfiffner (4). However, in the majority of cases the effects observed could be restored to normal by the administration of desoxycorticosterone or salt. Therefore an approach to the metabolic effects of cortisone must be such that disturbances due primarily to faulty electrolyte balance must be recognized and corrected.

For this reason we have based the following studies on the proposition that, if cortisone were involved in any system observable by way of the

¹ The term Cortone (Merck) will be used in this paper to identify a specific type of cortisone preparation, consisting of cortisone acetate formulated in suspending agents and containing 1.5 per cent benzyl alcohol as a preservative. The term cortisone will be used in a broader sense to indicate the free steroid, its esters, or whatever active substance it may be converted into in vivo.
homogenate technique, this system should alter on adrenalectomy (absence of cortisone plus the absence of other adrenal factors) and should be restored toward normal by treatment of the adrenalectomized animal with cortisone. We have used the rat as a test animal, attempting to correct for the salt effect in adrenalectomized rats by supplying 0.9 per cent NaCl rather than water, whether or not the animal received supplementary treatment with cortisone. The activities observed were compared to those of normal animals supplied with water rather than saline. While admittedly this is not an entirely complete picture of the possible variations in treatment, it is sufficient to reveal metabolic effects of cortisone demonstrable by the homogenate method. Using this type of approach we have found two effects of adrenalectomy and treatment with cortisone upon the metabolism of kidney homogenates. These are as follows: (1) Proline oxidation by kidney homogenates decreases when the animal is adrenalectomized and is restored to normal by treatment with cortisone. (2) The adenylic acid (or adenosinetriphosphate) requirement of homogenates is increased after adrenalectomy and restored to normal by treatment with cortisone. The increased adenylate requirement is reflected in the lowered rates of several reactions, activities of which are dependent upon adenylate.

Since these are two separate aspects of the effects of adrenalectomy and cortisone, we have divided the information into two papers.

Before considering proline oxidation in detail, it should be mentioned that with the exception of the effect of adenylic acid, to be described in Paper II, which indirectly affects some of the reactions listed, we have found no effect either of adrenalectomy or of cortisone treatment on the following reactions (the tissues studied are represented by K. = kidney, L. = liver, M. = muscle): α-ketoglutarate oxidase system (K., L.), oxalacetate oxidase (K., L., M.), succinic oxidase (K., L., M.), lactic dehydrogenase (K., L.), glutamic dehydrogenase (K., L.), acetoacetic oxidase (K., M.), octanoic acid oxidase (L.), transaminase (K., L., M.), and choline esterase (M.).

**Methods**

The rats, weighing approximately 150 gm., were adrenalectomized bilaterally in the laboratory of Dr. H. C. Stoerk, to whom we are greatly indebted for this material. After adrenalectomy, the animals were provided with a mixed adequate diet (except as specified) and with 0.9 per cent NaCl in place of drinking water. Treatment with cortisone consisted of the intramuscular injection of specified doses of Cortone, supplied as a single daily dose. Conventional manometric methods (18) were employed throughout. All homogenates were prepared in isotonic
(1.15 per cent) KCl according to the methods described by Potter and Schneider (18) using "10 per cent homogenates;" that is, 1 part of tissue to 10 parts of KCl. Isotonic sucrose homogenates gave identical results. Ammonia and carbon dioxide were determined as described elsewhere (18) and proline was determined colorimetrically on trichloroacetic acid filtrates by a slight modification of the ninhydrin method of Moore and Stein (19) with a Beckman spectrophotometer (at 440 mμ). Glutamate was determined by employing glutamic decarboxylase. In measuring oxygen uptake, a 6 minute equilibration period was used and measurements were begun 6 minutes after the ice-cold flasks, tissue, and reactants were placed in the Warburg bath at 37°.

Results

A typical curve for the oxidation of proline by kidney homogenates is given in Fig. 1; for the details of the exact conditions employed see the legend. With the first 20 minute period as the criterion of the rate of the reaction, results obtained with a larger series of animals are given in Table I. It is apparent that these results are statistically significant and that upon adrenalectomy the ability of a given quantity of kidney to oxidize proline was reduced by about one-half. Upon treatment with cortisone, the proline-oxidizing ability of the tissue was restored to normal (or in some cases, slightly above normal).

The mechanism of proline oxidation was shown to be essentially that described by Taggart and Krakaur (20), and no difference in the course of the reactions could be detected between the adrenalectomized and cortisone-treated systems.

Conditions Which Affect Proline Oxidation—By using the standard system, as indicated in Fig. 1, for measuring the rate of proline oxidation a study was made of the time after adrenalectomy required before proline oxidation was affected. The data, given in Fig. 2, show that by 2 to 3 days after adrenalectomy the decrease in ability to oxidize proline was virtually maximum and that it remained at the same level thereafter. 30 to 60 days after adrenalectomy the same low rate of proline oxidation was observed. If cortisone (at 1 mg. per day) was given to the adrenalectomized rats, the rate was maintained at the normal level. If the proline oxidation rate were allowed to decrease and cortisone treatment instituted at 1 week after adrenalectomy, approximately 7 days of treatment (at 1 mg. per day) restored full activity, but a detectable effect was noted after 2 days of treatment.

The amount of cortisone required for maintenance of activity after adrenalectomy is shown in Fig. 3, in which it appears that 0.5 mg. daily is an adequate amount. Throughout the entire study of proline oxidation,
various attempts were made to activate the homogenates from both the adrenalectomized and cortisone-treated rats by the addition \textit{in vitro} of cortisone acetate, free cortisone, and heat-killed homogenates from both adrenalectomized and cortisone-treated animals. In no case was an effect observed \textit{in vitro} (either stimulation or inhibition). Cortisone exerted its action only when supplied \textit{in vivo}. Treatment of the adrenalectomized
animal with desoxycorticosterone acetate did not prevent the loss of the homogenates' ability to oxidize proline.

Proline oxidation was also studied in tissue slices. All the slices were prepared with adequate precautions as to thickness, isotonicity, etc. The oxygen uptake at 3 hours for the slices not supplied proline and those given

![Graph](image1)

**Fig. 2.** Proline oxidation by kidney homogenates at intervals after adrenalectomy. Conditions of measurement as in Fig. 1.

![Graph](image2)

**Fig. 3.** Dosage of cortisone required for maintenance of proline oxidation system in adrenalectomized rats. The usual range indicates values found for a larger series of animals at 0 and 1 mg. of cortisone daily. All animals tested 3 days after adrenalectomy.
proline, which one would presume to be a measure of proline oxidation, is given in Table II. The straight line oxidation continued for several hours. Thus the tissue slices in Krebs-Ringer solution showed a decreased ability (of the order of 40 per cent) to oxidize proline when the slices had been obtained from an adrenalectomized animal. In the KCl-potassium phosphate buffer (sodium and calcium are omitted) the oxidation of proline by the slices obtained from the adrenalectomized animal is only 30 per cent of the normal (i.e. a decrease of 70 per cent in activity). The study of proline oxidation in tissue slices shows that, while the same effect which is observable in homogenates can also be demonstrated in the slice, the nature of the suspending medium is of great importance in determining the magnitude of the difference between the tissues from normal and adrenalectomized rats. We suggest that perhaps this is one of the reasons

**Table II**

<table>
<thead>
<tr>
<th></th>
<th>Medium</th>
<th>With proline</th>
<th>Without proline</th>
<th>Increase in oxygen due to proline</th>
<th>Per cent of normal increase in oxygen uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, Krebs-Ringer solution</td>
<td></td>
<td>706</td>
<td>410</td>
<td>296</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized, Krebs-Ringer solution</td>
<td></td>
<td>754</td>
<td>573</td>
<td>181</td>
<td>61</td>
</tr>
<tr>
<td>Normal, KCl-PO₄*</td>
<td></td>
<td>675</td>
<td>318</td>
<td>357</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized, KCl-PO₄</td>
<td></td>
<td>458</td>
<td>346</td>
<td>112</td>
<td>31</td>
</tr>
</tbody>
</table>

* Phosphate buffer at pH 7.4.

why there has been lack of agreement among investigators as to the effects of adrenalectomy on metabolism based on tissue slice techniques.

With the establishment of the empirical fact that, upon adrenalectomy, homogenates prepared from the kidney exhibit a lowered proline oxidation, it became of interest to examine the effects of various vitamin deficiencies upon the rate of proline oxidation, since several vitamins have been thought to act by influencing adrenal function (21-23). If a lowered proline oxidation were obtained, this would not provide evidence that the vitamin did act by influencing cortisone production in the adrenal, since the vitamins involved either in synthesizing the enzyme or in its activity are not known. But if a vitamin deficiency, which exhibits in physiological or other tests a similarity to the symptoms observed on adrenal insufficiency, did not decrease proline oxidation, the effect of the lack of the vitamin must be more complex than lack of cortisone. It should first be pointed out that the ability of the kidney to oxidize proline, or the difference between cortisone-treated and untreated adrenalectomized animals, is not dependent upon previous fasting of the animal. All of the data so far
presented are from rats fed *ad libitum* and food was available up to the moment that the animal was killed. Identical results, however, were obtained if the animals were fasted for an 18 to 24 hour period before killing. Therefore, any inanition or lack of appetite would not be expected to influence the proline oxidation reaction. The results obtained are given in Table III. It is apparent that the animals with various vitamin deficiencies do not lack the ability to oxidize proline and are therefore not lacking in cortisone. Whatever the effects of these vitamins may be in decreasing apparent adrenal function, they apparently do not eliminate cortisone. One further point requires consideration here. The proline oxidation effect is specific in the sense that it is evident with proline (but not with other amino acids oxidizable by rat kidney) and that cortisone but not desoxycorticosterone is active. The oxidation of proline in rat

**Table III**

Effect of Vitamin Deficiencies on Proline Oxidation by Kidney Homogenates

<table>
<thead>
<tr>
<th>Rat treatment</th>
<th>Q₀₂ on proline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>10</td>
</tr>
<tr>
<td>&quot; treated with cortisone</td>
<td>26</td>
</tr>
<tr>
<td>Vitamin B₄-deficient</td>
<td>30</td>
</tr>
<tr>
<td>Riboflavin-deficient</td>
<td>21</td>
</tr>
<tr>
<td>Thiamine-deficient</td>
<td>21</td>
</tr>
<tr>
<td>Pantothenic acid-deficient</td>
<td>27</td>
</tr>
<tr>
<td>Vitamin B₁₂-deficient</td>
<td>28</td>
</tr>
</tbody>
</table>

liver, however, was found to be (five in each group) for adrenalectomized rats, Q₀₂ = 16 ± 0.5, adrenalectomized and treated with cortisone, 17.5 ± 0.5. It is apparent that the proline oxidase of liver is much less affected (if at all) by adrenalectomy than that of the kidney. The relation of cortisone to proline oxidase thus has a further specificity in being related to cortisone in the kidney, but is apparently independent of cortisone in the liver.

These facts necessarily limit considerations of the significance of the relation between cortisone control of proline oxidation and the mode of action of cortisone in the body. While it is possible, at least in theory, that the derangement of the metabolism of but one amino acid could produce wide-spread and profound effects in metabolism, such a derangement would certainly be expected to be evident in all tissues of the body. It is, for the moment, difficult to see any particular relation of proline to the effects of cortisone observed by other techniques. There is, of course, the fact that, of all the proteins, only collagen and edestin contain much proline, but even this fact may bear no relation to the variations in proline.
metabolism. Therefore, at the moment it seems best to regard the proline oxidation reported here as a useful tool for the study of cortisone rather than as an attempt to assess its significance in the physiological rôle of cortisone in the body.

SUMMARY

Upon adrenalectomy, the ability of kidney homogenates to oxidize proline is decreased. This ability is restored to normal by cortisone treatment. The decreased proline oxidation is evident 2 to 3 days after adrenalectomy. The proline system may be restored by cortisone treatment, but as much as a week of continued treatment is necessary. The amount of cortisone required for maintenance of the proline system is 0.5 mg. daily. Treatment of the adrenalectomized animal with desoxycorticosterone did not maintain proline oxidase ability; treatment with cortisone or whole adrenal extract did. The differences in proline oxidation are evident in tissue slices as well as in homogenates. Various vitamin deficiencies did not decrease kidney proline oxidase. The effect observed seems to be peculiar to the kidney and is not evident in a comparison with liver proline oxidation.

BIBLIOGRAPHY

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