THE EFFECT OF CORTISONE UPON PROTEIN SYNTHESIS

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For some time, it has been known that the injection of adrenal cortex extracts or adrenal steroids containing oxygen substitutions at the C11 position augments the excretion of nitrogen in the urine of animals either fasting or feeding normally. Similar injections into adrenalectomized or hypophysectomized animals also raise the fasting nitrogen excretion above normal (1-3). On the contrary, the loss of cortical secretion is accompanied by a decreased urinary nitrogen excretion (3-9).

It was thought that finding the mechanism of the increased output of urinary nitrogen, following adrenal steroid therapy, might help to clarify the role of these steroids in protein metabolism. Cortisone (17α-hydroxy-11-dehydrocorticosterone) typically increases the urinary nitrogen excretion of rats and man. In 1942, Albright (10) suggested that the 11-oxygenated steroids perhaps were concerned with an antianabolic action rather than a catabolic one on protein. However, there are conflicting reports in the literature as to whether cortisone exerts this effect upon protein synthesis (11) or upon protein breakdown (12). The results obtained in the following experiments appear to confirm the Albright concept that one of the actions of the adrenal cortical hormone is to inhibit protein synthesis, although a lesser effect upon protein catabolism cannot be overlooked.

When an amino acid is fed to an animal, it mixes with the other constituents of the metabolic pool and part of its nitrogen may be used for protein synthesis while another part is converted to excretory products. The more efficient the utilization of the amino acid for protein synthesis, the less of its nitrogen will be found in the urine. Recently, Sprinson and Rittenberg (13) have shown that, by feeding a tracer dose of an isotopic amino acid (N15-glycine) and by determining the amount of its nitrogen excreted in the urine, they could calculate the rate of protein synthesis. This technique has accordingly been employed in both normal and adrenalectomized rats, some of which had received cortisone, in an attempt to determine whether cortisone affected the rate of protein synthesis.

Experiment 1—White male rats of the Wistar strain were used. 1 month prior to their use, half had been adrenalectomized and a sham operation was performed on the other half. All animals received 1 per cent sodium chloride in their drinking water. The treated animals received 3 mg. of
cortisone acetate subcutaneously every day for 5 days. During the test period, all animals were given slightly less food than they would eat *ad libitum*. On the 4th day all the animals received by stomach tube 25 mg. of glycine containing 32 atom per cent excess N\textsuperscript{15}. Urines, collected quantitatively under toluene over a 48 hour period, were analyzed for total nitrogen, urea nitrogen, ammonia nitrogen, and N\textsuperscript{15}.

### Table I

*Data in Experiment 1 on Protein Synthesis in Metabolism of Rats*

<table>
<thead>
<tr>
<th></th>
<th>Adrenalectomized + cortisone</th>
<th>Adrenalectomized control</th>
<th>Sham operation + cortisone</th>
<th>Sham control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat 6</td>
<td>192</td>
<td>212</td>
<td>220</td>
<td>222</td>
</tr>
<tr>
<td>Rat 8</td>
<td>220</td>
<td>213</td>
<td>208</td>
<td>204</td>
</tr>
<tr>
<td>Rat 7</td>
<td>220</td>
<td>222</td>
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<td>211</td>
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<td>Rat 9</td>
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<td>220</td>
<td>211</td>
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<tr>
<td>Rat 12</td>
<td>531</td>
<td>326</td>
<td>538</td>
<td>539</td>
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<tr>
<td>Rat 15</td>
<td>539</td>
<td>405</td>
<td>648</td>
<td>644</td>
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<tr>
<td>Rat 17</td>
<td>571</td>
<td>648</td>
<td>180</td>
<td>180</td>
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<tr>
<td>Initial weight</td>
<td>192</td>
<td>212</td>
<td>220</td>
<td>222</td>
</tr>
<tr>
<td>Final weight</td>
<td>222</td>
<td>211</td>
<td>208</td>
<td>204</td>
</tr>
<tr>
<td>Weight changes, gm. in 5 days</td>
<td>-28</td>
<td>-34</td>
<td>-9</td>
<td>-2</td>
</tr>
<tr>
<td>Food intake, gm. in 5 days</td>
<td>35</td>
<td>36</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Urea N, 48 hrs., mg</td>
<td>531</td>
<td>326</td>
<td>538</td>
<td>539</td>
</tr>
<tr>
<td>Total N, 48 hrs., mg</td>
<td>701</td>
<td>405</td>
<td>648</td>
<td>644</td>
</tr>
<tr>
<td>N\textsuperscript{15}, 48 hrs., <em>% dose excreted</em></td>
<td>61</td>
<td>60</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>$S_K$†</td>
<td>1.09</td>
<td>1.39</td>
<td>2.20</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.10</td>
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<td>1.56</td>
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<td></td>
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<td></td>
<td>1.62</td>
</tr>
</tbody>
</table>

* P for the difference between the cortisone-dosed and control animals = <0.005.
† Incomplete urine; part of the 1st day’s collection was spilled.
‡ $S_K$, rate of protein synthesis per kilo of rat.

### Results

Although all the animals ate approximately the same amount of food during the test period (Table I), the animals receiving cortisone lost considerably more weight than their respective controls. The loss in weight observed in these animals cannot be accounted for by a decreased food intake but may be the result either of an increased breakdown of tissue or of a decrease in the utilization of the ingested food for tissue synthesis.

The urinary nitrogen values (Table I) show the pronounced effect of cortisone in increasing nitrogen excretion. Moreover, the adrenalectomized controls retain somewhat more nitrogen than do the sham controls. Since urea, the main excretory product of protein metabolism, is formed in the liver, it appears that the administration of cortisone results in an increased metabolic activity of this organ. It has been reported that adrenal steroids do increase the arginase activity of the liver (14).

\[1\] Cortone, Merck.
An examination of the isotope data (Table I) shows that the cortisone-treated animals excrete much more of the ingested isotope than do their respective controls. It is possible that these animals either cannot utilize the dietary protein for synthesis as efficiently as do the normal rats or are catabolizing tissue protein at a greater rate than normal.

Upon calculating the rate of protein synthesis (S_k) (Table I) by the method of Sprinson and Rittenberg (13), it was found that the administration of cortisone to either normal or adrenalectomized rats resulted in a decrease in the over-all rate of protein synthesis as compared to their controls. The rate of protein synthesis of the adrenalectomized controls appeared to be slightly greater than that of the sham controls. However, an analysis of variance (15) revealed differences of dubious significance between adrenalectomized and normal rats.

The data suggest that the dietary nitrogen is converted preferentially into excretory products rather than into tissue protein by the animals that had received cortisone.

Experiment 2—To investigate the effect cortisone has upon tissue utilization of dietary amino acids, groups of rats similar to those in Experiment 1 were set out, except that the animals receiving cortisone were on test for 10 days. On the 9th and 10th days, all the rats received 1 mm of isotopic glycine containing 32 atom per cent excess N\textsubscript{15} per 100 gm. of body weight. Urines were collected quantitatively under toluene on the 10th and 11th days and were analyzed for urea nitrogen, ammonia nitrogen, and N\textsubscript{15} in the usual manner. 24 hours after the last dose of glycine, the animals were sacrificed by decapitation and serum was collected. The carcass (everything except skin and viscera) and the liver were ground separately in a Waring blender in 70 per cent alcohol and then extracted in a Soxhlet extractor with 95 per cent alcohol for 8 hours, followed by ether extraction for 3 hours. All the extracts were evaporated to dryness and taken up in water, centrifuged when necessary, and made up to a suitable volume from which aliquots were taken for analyses for non-protein nitrogen, creatinine, and N\textsubscript{15}. The defatted tissues were air-dried and then dried over P\textsubscript{2}O\textsubscript{5} in a desiccator to constant weight. Suitable aliquots were taken for total nitrogen and N\textsubscript{15} determination.

Results

As in the first experiment, the loss in weight of the animals receiving cortisone cannot be correlated with any decrease in food intake. During the last 4 days of the experiment, all the animals received 10 gm. of diet daily; yet the animals given cortisone lost considerably more weight than the controls.

The urinary nitrogen excretion also parallels that in Experiment 1, there
being a marked increase in the excretion of urea nitrogen, total nitrogen, and $N^{15}$ by the cortisone-treated animals. The $P$ for difference between cortisone-dosed rats and controls again is $<0.005$. Again, the data suggest that cortisone results in an impairment in the utilization of the dietary protein.

Reference to the data in Table II reveals that the carcass tissues of the cortisone-treated animals, in fact, do contain less $N^{15}$ than those of the control. The lower $N^{15}$ content of these tissues would indicate that in cortisone treatment the utilization of amino acids for protein synthesis does occur, but with a lowered efficiency. It is also possible that replacement of amino acids within the protein may occur but that no new protein formation may take place.

The situation in the liver is exactly the opposite of that found in the rest of the carcass (Table III). Here the livers of the cortisone-treated rats are much larger and contain more protein nitrogen and $N^{15}$. This increase in protein nitrogen and $N^{15}$ clearly indicates that these livers are actually synthesizing more protein than the controls. Similar findings have been obtained by Silber and Porter.²

Another indication of an increased metabolism of the liver is the total "creatinine" values as determined by the Jaffe reaction. The livers of the control animals contain about one-half as much as do those of the cortisone-

² Silber, R. H., and Porter, C. C., unpublished data.
dosed rats. Recently, Umbreit and Tonházy (16) have shown that this increase in Jaffe-positive material is due to an increase in the deamination of methionine, the resulting keto acid reacting similarly to creatinine in the Jaffe test.

Since the liver is the site of albumin formation, a further indication of its heightened metabolism is the increase of both serum protein and its N\textsuperscript{15} content.

**Table III**

<table>
<thead>
<tr>
<th></th>
<th>Protein N</th>
<th>Non-protein N</th>
<th>Serum</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(\text{g}m)</td>
<td>(\text{mg})</td>
<td></td>
</tr>
<tr>
<td><strong>Adrenalectomized + cortisone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A</td>
<td>2.37</td>
<td>296</td>
<td>6.27</td>
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<tr>
<td>17A</td>
<td>2.42</td>
<td>291</td>
<td>6.14</td>
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<tr>
<td><strong>Adrenalectomized control</strong></td>
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<tr>
<td>4</td>
<td>1.56</td>
<td>234</td>
<td>4.27</td>
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<tr>
<td>27</td>
<td>1.45</td>
<td>214</td>
<td>4.43</td>
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<td><strong>Sham + cortisone</strong></td>
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<td>33</td>
<td>2.03</td>
<td>268</td>
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<td>2.54</td>
<td>318</td>
<td>7.75</td>
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<td><strong>“ control</strong></td>
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<tr>
<td>53</td>
<td>1.64</td>
<td>242</td>
<td>5.20</td>
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<tr>
<td>55</td>
<td>1.76</td>
<td>264</td>
<td>5.36</td>
</tr>
</tbody>
</table>

* \(P\) for difference between cortisone-dosed and control animals = <0.05.
† \(P\) for difference between cortisone-dosed and control animals = <0.005.

**DISCUSSION**

Nitrogen balance studies have been used successfully for many years to evaluate the net protein metabolism of animal and man. Unfortunately, the data obtained from such studies give no indication as to the source of the excreted nitrogen. The isotope technique, as used by Sprinson and Rittenberg (13), now enables one to ascertain whether or not the urinary nitrogen is endogenous or exogenous. The data (Table I) from Experiment 1 show that the rats which received cortisone excreted almost twice as much of the ingested isotope as did their respective controls. This would
indicate that these animals do not utilize the exogenous nitrogen as efficiently as do the untreated rats. The calculated rate of protein synthesis of these animals shows this to be the case. Cortisone treatment would appear to impair protein synthesis by interfering with the formation of new protein.

The decrease in the rate of protein synthesis found by calculation in Experiment 1 is confirmed by the results of Experiment 2. Here it can be seen (Table II) that the actual amount of isotope fed which was incorporated into the carcass tissue, that is everything except skin, viscera, and non-protein nitrogen of the cortisone-treated animals, is considerably less than that found in the control rats. The data obtained from the liver are diametrically opposed to those of the carcass. Since the liver is relatively small compared to the carcass, changes in this organ will be overshadowed by those of the carcass. Thus the use of urinary excretion data to evaluate the status of protein metabolism is valid only when the over-all picture is concerned.

From these experiments, it appears that one of the actions of the adrenal hormone is to regulate protein synthesis. In the dynamic state, there is a continual breakdown of protein to amino acids, followed by a resynthesis.

\[
\begin{align*}
\text{Protein} & \quad \xrightarrow{\text{breakdown}} \quad \text{amino acids} \\
\text{protein synthesis} & \quad \xrightarrow{\text{amino acids}} \quad \text{Protein}
\end{align*}
\]

Since the uptake of the isotope by the protein is less in the treated animals, it is clear that Reaction A is hindered. The possibility that an increase in the rate of Reaction B is the deciding factor can be ruled out since the N\(^{15}\) incorporated is greatly diluted with normal nitrogen, and even a great increase in the degradative rate would merely result in a very small fraction of the isotope being excreted in 48 hours. Further evidence against the role of cortisone as an accelerator of protein catabolism is its failure to effect the rate of disappearance of antibody protein (17). This hypothesis that one of the actions of cortisone is to limit protein synthesis is supported by other evidence. Clinically and experimentally, it has been shown that the administration of cortisone markedly retards the healing of wounds (18, 19). Layton has shown in vitro that cortisone interferes with the incorporation of sulfur in chondroitin sulfuric acid (20). Other investigators have also shown a decreased uptake of C\(^{14}\)-glycine by the muscles and kidneys of rats treated with adrenocorticotropic (21). A catabolic effect of cortisone cannot be completely ruled out by these experiments. However, from the data presented here the antianabolic effect of this hormone appears to be of greater significance in the regulation of protein metabolism.
I. CLARK

The increased metabolism of the liver is most probably a compensatory reaction. The liver once more plays its familiar rôle as a "detoxicating" agent by metabolizing any excess of amino acids and other nitrogen compounds that may have resulted from the impairment of protein synthesis by cortisone.

Another interesting finding revealed by the data is that, when there is a decrease in the synthetic activity of a tissue, there is a marked drop in the amount of non-protein nitrogen and N\textsuperscript{15} of this tissue (Table II). On the other hand, if the synthetic activity of a tissue is heightened, there appears to be an increase in the non-protein nitrogen and N\textsuperscript{15} of this fraction (Table III). A comparison of the non-protein nitrogen of different organs of animals under various treatment may reveal the metabolic status of the particular organ in question. Experiments are under way to ascertain whether this is generally true.

The author wishes to express sincere thanks to Mr. Edward Lehman of the Merck Institute for valuable technical assistance, to Mr. Irving Sucher for the isotopic analyses, and to Professor David Rittenberg, both of the College of Physicians and Surgeons, Columbia University, for valuable discussions and advice concerning this work.

SUMMARY

1. Evidence is presented that cortisone causes an increase in urinary nitrogen because it acts as an inhibitor or regulator of protein synthesis.

2. The validity of using urinary nitrogen data for calculating the status of protein synthesis in the rat has been confirmed by tissue analyses.

3. It is suggested that, during cortisone treatment, the liver acts as a detoxicating agent by preventing the accumulation of nitrogenous substances not elsewhere utilized by the body.

4. Evidence is presented that an increased anabolic activity of an organ is accompanied by an increase in its non-protein nitrogen. Also, increased catabolic activity of an organ is characterized by a decrease in its non-protein nitrogen.

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