FACTORS INFLUENCING THE EXCRETION OF TAURINE IN IRRADIATED RATS WITH PARTICULAR REFERENCE TO THE ADRENAL GLANDS

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Kay and coworkers (1) have shown an increased excretion of taurine by rats after total body x-irradiation. It has been demonstrated previously (2–4) that methionine, cysteine, and glutathione are precursors of taurine. In view of the well established effect of irradiation on sulfhydryl groups described by Barron and his associates (5–7), it seems probable that the increase in the excretion of taurine is due to increased oxidation of sulfhydryl compounds. This view is strengthened by the report of Aebi et al. (8), who failed to find an additional increase in taurine excretion by rats that had been injected with cysteine and then subjected to 500 r. of total body radiation. The rationale for this was that the injected cysteine spared the previously existing supply of sulfhydryl groups.

Since both the thymus and spleen may be destroyed by irradiation, it was of interest to determine whether removal of these tissues would alter the taurine excretion values after exposure to x-radiation.

It has also been established by Patt and others (9–12) that total body irradiation induces a fall in the ascorbic acid and cholesterol content of the adrenal cortex. Recently it has been shown by Pentz and Hasterlik (13) that the diuresis which occurs in rats after total body irradiation is dependent upon the adrenal cortex. It was therefore pertinent to examine the excretion of taurine by adrenalectomized animals after irradiation and to investigate the relationship between taurine excretion and urine volume.

Methods

Female Sprague-Dawley rats were used for these experiments. They were maintained at a temperature of 25.5° ± 1°. Except during urine collection periods, they were given Rockland mouse pellets and water *ad libitum*. Adrenalectomized animals were given 1 per cent sodium chloride as drinking water.

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Rats were grouped by randomizing on a body weight basis. Collection of urine free from extraneous material was accomplished by placing the animals in metabolism cages1 (two per cage) for 6 hours during the day. During this time they were fasted but had free access to drinking fluid unless otherwise stated. A period of training to the regimen of at least 4 days always preceded irradiation (except as noted). Animals continued to gain in body weight under the experimental conditions. Urine was collected under toluene in 50 ml. graduates placed under the collection tubes of the metabolism cages. The daily collections of urine from all animals of each group were pooled for analysis. Irradiation was carried out early in the morning, and the first urine collection period was 6 hours immediately thereafter.

Irradiation was achieved by placing unanesthetized rats in individual sections of a circular aluminum carrier that was perforated uniformly for maximal exposure. The carrier was placed on a slowly rotating table during irradiation. Radiation was generated by a 250 kvp. Maxitron machine, operating at 30 ma. The target to skin distance was 92 cm., and the exposure rate was approximately 30 r. per minute. Control non-irradiated animals were subjected to the same manipulations.

Urine samples were separated from the toluene and filtered immediately before analysis. Taurine determinations were carried out by the method of Pentz and coworkers (14). Specimens of urine were hydrolyzed by adding an equal volume of HCl (concentration) and autoclaving for 5 hours at 15 pounds and 250° (14).

Paper chromatography was of the descending two-dimensional type, with Whatman No. 1 filter paper sheets 11 1/2 X 13 1/2 inches. The first solvent was 80 per cent phenol saturated with water and the second 2,4-lutidine, 65 per cent, and water, 35 per cent.

Adrenalectomy was performed on rats under ether anesthesia by way of bilateral incisions, and all operation sites were inspected at autopsy for accessory adrenal tissue.

Results

Four groups of ten animals each, having an average body weight of 145 gm. at the time of irradiation, were divided so that two groups received 400 r. and two groups received 0 r. Drinking water was withheld from each of one irradiated and one control group for the 6 hour collection period immediately after irradiation because polyuria does not occur during the

1 Manufactured by the Acme Sheet Metal Works, Chicago. An additional screen of 8 mesh, 0.027 wire was placed horizontally in the collection funnel 1 inch below the floor of the cage. A cylinder of similar wire screen was fitted into the delivery tube of the collection funnel so that approximately 1 inch protruded above the tube. Urine so collected from healthy rats is satisfactorily free from solid material.
first collection period under these circumstances (15, 16). Taurine excretion values for unhydrolyzed urine specimens for a successive 6 hour collection period, both before and after irradiation, are shown in Fig. 1.

It was pertinent to examine the effect of other dosages of x-ray on taurine excretion under similar conditions. Consequently, four groups of ten animals each (average weight 163 gm.) were exposed respectively to 0, 200, 400, or 600 r., and again drinking water was withheld during the collection period immediately after irradiation. Taurine excretion values for unhydrolyzed urine specimens are shown in Fig. 2 and for hydrolyzed aliquots of the same specimens in Fig. 3.

![Fig. 1. Taurine content of unhydrolyzed urine specimens from rats in relation to total body irradiation with and without drinking water immediately after exposure. Controls, 0 r. with water, open circle with dashed line; exposed, 400 r. with water, solid circle with dashed line; controls, 0 r. without water for 6 hours, open circle; exposed, 400 r. without water, solid circle. All groups were composed of ten animals each.](image-url)

In order to explore changes in taurine excretion values in adrenalectomized animals, ten groups of six animals each were treated in the following manner. Four groups were adrenalectomized, four groups were sham adrenalectomized, and two groups were unilaterally nephrectomized. Two adrenalectomized groups, two groups sham adrenalectomized, and one nephrectomized group were irradiated with 400 r. 13 days after surgery. The remaining groups were not irradiated. Taurine analyses were performed as in the previous experiments. Of the five groups that originally served as controls, one adrenalectomized group, one sham adrenalectomized group, and the unilaterally nephrectomized group were irradiated with 400 r. 31 days after surgery. One adrenalectomized group and one sham adrenalectomized group served as controls. The results of the taurine analyses in similarly treated groups were so nearly alike that they have been averaged and are shown in Fig. 4. The bars indicate the range of the two or three values from which the averages were derived.
Adrenalectomized animals begin to die on the 3rd and 4th day after exposure to 400 r. Death is sometimes preceded by diarrhea, and urine specimens were withheld from the pools when it was impossible to prevent contamination of the urine with fecal matter.

**Fig. 2.** Taurine content of unhydrolyzed urine specimens from normal rats that received 200 r., open triangle; 400 r., solid circle; 600 r., solid triangle; and 0 r., open circle with dashed line, of total body irradiation. Drinking water was withheld for 6 hours after exposure. All groups were composed of ten animals each.

**Fig. 3.** Taurine content of hydrolyzed urine specimens from normal rats that received 200 r., open triangle; 400 r., solid circle; 600 r., solid triangle; and 0 r., open circle, dashed line, of total body irradiation. Drinking water was withheld for 6 hours after exposure. All groups were composed of ten animals each.
Taurine values in the adrenalectomized groups were so high that it was thought advisable to check the analyses by paper chromatographic examination in case the limits of the specificity of the method (14) had been exceeded. These tests were performed by using the filtrate from Dowex-treated urine samples along with appropriate positive taurine controls. In each case a single spot of taurine was obtained.

Adrenalectomized rats along with appropriate controls subjected to sham operation were exposed to 400 r. and 0 r. in three subsequent experiments with virtually identical results.

Data for the concentration of taurine in urine are shown in Table I.
**Table I**

Concentration of Taurine in Unhydrolyzed Urine from Adrenalectomized, Sham Adrenalectomized, and Unilaterally Nephrectomized Control and Total Body X-radiated Rats, and from Normal Rats Receiving No Drinking Water for 6 Hours Immediately After Irradiation

The values are given as mg. per ml. per rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Radiation (r.)</th>
<th>-2 days</th>
<th>-1 day</th>
<th>X-ray day</th>
<th>+1 day</th>
<th>+2 days</th>
<th>+3 days</th>
<th>+4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomy</td>
<td>400</td>
<td>0.31</td>
<td>0.30</td>
<td>0.42</td>
<td>2.95</td>
<td>1.5</td>
<td>1.45</td>
<td>0.85</td>
</tr>
<tr>
<td>&quot;</td>
<td>0</td>
<td>0.32</td>
<td>0.56</td>
<td>0.30</td>
<td>0.55</td>
<td>0.15</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>Adrenalectomy (sham operated)</td>
<td>400</td>
<td>0.62</td>
<td>0.40</td>
<td>0.25</td>
<td>0.32</td>
<td>0.32</td>
<td>0.20</td>
<td>0.43</td>
</tr>
<tr>
<td>Unilateral nephrectomy</td>
<td>400</td>
<td>0.60</td>
<td>0.75</td>
<td>0.90</td>
<td>0.81</td>
<td>0.64</td>
<td>0.72</td>
<td>0.60</td>
</tr>
<tr>
<td>&quot;</td>
<td>0</td>
<td>0.50</td>
<td>0.62</td>
<td>0.20</td>
<td>0.24</td>
<td>0.18</td>
<td>0.15</td>
<td>0.61</td>
</tr>
<tr>
<td>Water withheld</td>
<td>0</td>
<td>0.29</td>
<td>0.65</td>
<td>0.52</td>
<td>0.45</td>
<td>0.45</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>200</td>
<td>0.35</td>
<td>1.31</td>
<td>0.59</td>
<td>0.33</td>
<td>0.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>400</td>
<td>0.23</td>
<td>1.00</td>
<td>0.42</td>
<td>0.46</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>600</td>
<td>0.40</td>
<td>0.93</td>
<td>0.46</td>
<td>0.74</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The bold-faced type is used for emphasis.

**Table II**

Taurine Excretion As Determined by Analysis of Unhydrolyzed Urine Specimens from Control Rats and Rats Having Thymus and Spleen Removed in Relation to Total Body X-radiation

The values are given as mg. per 100 gm. per 6 hours.

<table>
<thead>
<tr>
<th>Group</th>
<th>Radiation (r.)</th>
<th>-2 days</th>
<th>-1 day</th>
<th>X-ray day</th>
<th>+1 day</th>
<th>+2 days</th>
<th>+3 days</th>
<th>+4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 (6)*</td>
<td>0.40</td>
<td>0.55</td>
<td>0.35</td>
<td>0.51</td>
<td>0.65</td>
<td>0.65</td>
<td>0.25</td>
</tr>
<tr>
<td>&quot;</td>
<td>400 (8)</td>
<td>0.35</td>
<td>0.78</td>
<td>0.62</td>
<td>2.18</td>
<td>0.82</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Thymus removed</td>
<td>400 (8)</td>
<td>0.32</td>
<td>0.37</td>
<td>1.10</td>
<td>1.80</td>
<td>2.74</td>
<td>0.31</td>
<td>0.20</td>
</tr>
<tr>
<td>Spleen</td>
<td>400 (8)</td>
<td>0.50</td>
<td>0.78</td>
<td>1.10</td>
<td>2.10</td>
<td>0.76</td>
<td>0.46</td>
<td>0.32</td>
</tr>
<tr>
<td>Thymus and spleen removed</td>
<td>400 (9)</td>
<td>0.30</td>
<td>0.34</td>
<td>1.03</td>
<td>1.96</td>
<td>0.76</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>Spleen (sham operated)</td>
<td>400 (6)</td>
<td>0.45</td>
<td>0.43</td>
<td>0.92</td>
<td>2.01</td>
<td>0.76</td>
<td>0.30</td>
<td>0.60</td>
</tr>
<tr>
<td>Thymus and spleen (sham operated)</td>
<td>0 (6)</td>
<td>0.33</td>
<td>0.42</td>
<td>0.45</td>
<td>0.72</td>
<td>0.55</td>
<td>0.65</td>
<td>0.42</td>
</tr>
</tbody>
</table>

* The figures in parentheses indicate the number of animals in each group, and the bold-faced type is used for emphasis.
At the time of irradiation all the animals weighed 160 ± 10 gm., and the figures are therefore comparable from group to group.

To examine the effects of removal of the thymus and spleen on taurine excretion in the rat, groups of animals that had either one or both of these tissues removed, along with appropriate controls, were exposed to 400 r. of x-radiation. Taurine excretion values for the various groups are shown in Table II.

Examination of the data makes it abundantly clear that removal of the thymus and the spleen from rats before irradiation does not reduce the excretion of taurine by these animals.

DISCUSSION

Normal values for the excretion of taurine by the rat have been published by Kay and his associates (1, 17), Awapara (18), Wu (19), and Aebi et al. (8), all of whom used paper chromatographic methods. By applying a factor of 4 to normal values found for the 6 hour collection period in the present experiments, the average value found was 2.0 mg. per 100 gm. per 24 hours. This is higher than some of the reported values and considerably lower than others.

Portman and Mann (20) have shown that prefeeding animals a diet low in organic sulfur results in the retention of taurine in the tissues and a decrease in its excretion in the urine. Neither in the above experiments nor in those reported by others has a careful control of organic sulfur intake been exercised. It is therefore quite possible that this factor may vary from laboratory to laboratory and from experiment to experiment. Such considerations may account in part for the difference in 24 hour excretion values that have been reported. The data for the hydrolyzed urine analyzed in the experiment in which increasing amounts of radiation were administered suggest that a quantitative relationship between quantity of radiation and taurine excretion might obtain if factors such as dietary intake of organic sulfur, collection of complete 24 hour urine specimens, etc., could be controlled. This suggestion is further supported by the data in Table II, where it is evident that the level of taurine excreted by all groups in response to 400 r. is remarkably consistent.

Examination of Fig. 1 reveals that there is a tendency for the excretion of taurine to parallel that of urine. However, the data in Table I show that this tendency does not always appear. Under both of the two conditions shown, namely that of the irradiation of adrenalectomized animals and that of withholding drinking water from irradiated normal animals, the taurine concentration of urine increases.

*Surgery was performed by the Hormone Assay Laboratories, Chicago.
Wu (19) has shown that continuously fasted rats excrete increasingly larger amounts of taurine in the urine as fasting progresses. Both Aebi and his associates (8) and Awapara (18) have demonstrated that the administration of cysteine increases the excretion of taurine. Awapara (18) and Wu (19) have been able to show a rise in the muscle content of taurine in fasting rats. As Wu has pointed out, this suggests that, as fasting progresses, the normal course of the oxidative processes of the sulfur-containing amino acids may be impaired, and the conversion of cysteine to taurine becomes predominant.

According to Selye (21), fasting is a stress in which the adrenal cortex undergoes particularly rapid and intense hypertrophy. This phenomenon is considered generally to be associated with an increased production of cortical hormones. The taurine excretion data for fasted rats published by Wu (19) revealed an increasing amount of taurine being excreted. This loss progresses during a 9 day fast to 10 times the amount of the control values. In view of the above findings, it is difficult to interpret the high taurine excretion values reported here for adrenalectomized animals after exposure to radiation. Cursory examination seemed to indicate that adrenal hormones are effective in either inhibiting or reversing oxidative processes which result from irradiation. Should this subsequently prove to be the case, the experiments of Wu (19) are probably best interpreted in terms of adrenal cortical exhaustion.

Since irradiation is considered to have both a direct and an indirect effect (via the adrenals) on lymphoid tissues (22, 23), the results of the experiment in which the two largest masses of lymphoid tissue, the thymus and the spleen, were removed are of some interest. It would not be surprising if the transitory rise in taurine excretion that occurs in normal animals on the day after irradiation were associated with known tissue breakdown. If this were so, it would be expected that removal of radiation-sensitive tissues before exposure would reduce or eliminate the rise in taurine excretion after irradiation. Examination of the data in Table II shows that this was not the case. If the rise in taurine excretion after total body exposure to x-ray represents lymphoid tissue breakdown, its origin must be the lymph nodes and circulating lymphocytes. This seems unlikely, however, in view of the much smaller total mass of lymphoid tissue available for destruction and the consistent quantitative data of Table II.

The catabolic effects of adrenal cortical hormones on protein metabolism are well established. However, Friedberg and Greenberg (24) were able to show that the hypoaminoacidemia after adrenalectomy was accompanied by a rise in the free amino acid level of the muscle. Awapara (18) reports a small increase in taurine content of muscle in adrenalectomized rats.
These, as well as the presently reported observations, all emphasize the fact that the functions of adrenal cortical hormone in relation to the metabolism of the sulfur-containing amino acids have not been elucidated.

**SUMMARY**

1. Changes in the taurine content of urine from rats receiving various levels of total body irradiation under conditions of restricted and unrestricted water intake are reported. Normal rats exposed to 600 r. showed an increase in taurine excretion of 3 times that of non-irradiated controls on the day after exposure when water intake was restricted for 6 hours immediately after irradiation. 48 hours after exposures up to 600 r., the taurine excretion levels had returned to normal values.

2. Under conditions of restricted water intake, the concentration of taurine in the urine of irradiated rats frequently increased.

3. The excretion of taurine by adrenalectomized rats after exposure to 400 r. was double that of unilaterally nephrectomized and control animals subjected to sham operation receiving the same amount of x-radiation. Taurine excretion did not return to normal levels in the adrenalectomized animals.

4. Removal of the thymus and the spleen did not reduce the amount of taurine excreted upon total body exposure to 400 r. as compared with normal and control animals subjected to sham operation.

**BIBLIOGRAPHY**

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