Factors Influencing Serum Copper and Ceruloplasmin Oxidative Activity in the Rat

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ABSTRACT

MEYER, B. J., A. C. MEYER AND M. K. HORWITT. Factors influencing serum copper and ceruloplasmin oxidative activity in the rat. Am. J. Physiol. 194(3): 581-584. 1958.—The effect of turpentine, estradiol benzoate and epinephrine hydrochloride on the copper concentration and paraphenylene-diamine oxidase activity of serum were studied in the male albino rat. All three of these substances raised the serum copper level and paraphenylene-diamine oxidase activity in both ‘control’ and ‘adrenalectomized’ animals, turpentine being the most active. The average serum copper level is approximately the same for normal humans and rats, while the phenylenediamine oxidase activity of normal human serum is about 2-3 times as high as that of rat serum. The data are discussed with relation to the known facts about serum copper in normal subjects and mental patients.

Dietary intake, tissue stores and excretory mechanisms are important in the regulation of serum copper concentration, but we know relatively little about the physiological control of these factors. In contrast with serum iron, the serum copper concentration is kept surprisingly constant in the normal animal and human being (1-3). In man, as well as in the albino rat, 95% or more of the serum copper is bound to a protein, ceruloplasmin, which behaves like a serum globulin (4, 5). In vitro, ceruloplasmin exhibits weak oxidative activity towards a variety of artificial substrates, including paraphenylene-diamine (ppd), adrenaline, ascorbic acid and dopa (6, 7). However, apart from suggestive evidence, it has not been proven that ceruloplasmin has any enzymatic function in the intact organism. Even if we accept the hypothesis that it is an enzyme, we still know nothing about its normal substrate.

Blood ceruloplasmin levels are highly sensitive to stress conditions (8-11). There is evidence that the endocrines are intimately related to the control of the serum ceruloplasmin concentration. Females have a higher ceruloplasmin level than males (2), and during pregnancy this concentration increases (9, 10, 12). Recently it was reported that estrogen increased the serum copper concentration in human subjects (13) and hypercupremia has been noted in patients with Addison’s disease (8, 14). However, corticotropin and cortisone did not influence the hypercupremia produced by turpentine in intact rats (15). It has been suggested that the plasma copper concentration depends upon the functional state of the thyroid gland, as thyrotoxicosis raises the blood copper level (8, 9). However, hypothyroidism is associated with normal plasma copper concentrations (8, 9, 16) and no significant correlation was noticed between serum copper concentration and basal metabolic rate (3, 16).

This study is an attempt to determine whether the adrenals play a role in the control of the serum copper level.

EXPERIMENTAL

Male rats of the Sprague-Dawley strain, weighing 300-400 gm were used in the present study. All animals were fed a stock diet of Purina laboratory chow ad libitum until they were killed. The animals were divided into...
two main groups. Animals in the first group were adrenalectomized under ether anesthesia, whereas those in the second group were treated similarly except that the adrenal glands were left intact (sham-operated animals). A solution of 0.9% saline plus 10% sucrose was substituted for drinking water for all animals. Injections of test materials were started 14 days after operation. Both control and experimental animals were weighed immediately before surgery, at the beginning of the period of treatment and on the day of killing. Animals whose wounds did not heal completely within 14 days after surgery, or did not regain their preoperative weight, were discarded. Each rat was kept in a separate cage at a room temperature of 80°F.

At the end of the experiment the animals were anesthetized with a single dose of 70 mg sodium amytaljrat dissolved in 2.5 ml of distilled water injected subcutaneously. Blood samples were drawn from the abdominal aorta.

Serum copper was determined by the method of Gubler et al. (17), and the phenylenediamine oxidative activity of the serum by the method described by Ravin (18). The latter method evaluates oxidative activity by measuring the increase in relative optical density of p-phenylenediamine hydrochloride solutions after incubating with serum at pH 6, at 37°C for 60 minutes. Readings were made in a Beckman model B spectrophotometer at 525μm through 10-mm light paths. The paraphenylendiamine oxidative activity of the serum is ordinarily regarded as a function of its ceruloplasmin content (3).

Effects of the following substances were studied: 1) estradiol benzoate (Dienormon benzoate, Organon) was diluted in sterile oil of sesame so that the required daily dosage of 0.05 mg estradiol benzoate was present in 0.2 ml oil of sesame. Injections were given intramuscularly, once daily, except on Sundays. Sham-operated control animals were treated similarly with 0.2 ml oil of sesame. 2) Turpentine (rectified oil of turpentine,

### Table 1. Factors Affecting Copper Levels and Oxidative Activity of Rat Serum

<table>
<thead>
<tr>
<th>Treatment: (Injections)</th>
<th>Saline Solution</th>
<th>Oil of Sesame</th>
<th>Turpentine</th>
<th>Estradiol Benzoate in Oil of Sesame</th>
<th>Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham-Operated Rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rats</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Weights (gm) when operated</td>
<td>320±11.1</td>
<td>333.4±23.5</td>
<td>333±18.4</td>
<td>109.5±13.1</td>
<td>380±19.5</td>
</tr>
<tr>
<td>Weights (gm) when killed</td>
<td>380±37.1</td>
<td>395.2±26.0</td>
<td>379.0±13.6</td>
<td>118.0±6.9</td>
<td>353.0±33.8</td>
</tr>
<tr>
<td>Serum copper μg/100 ml</td>
<td>109.5±13.1</td>
<td>118.0±6.9</td>
<td>187.2±12.3</td>
<td>141.0±6.9</td>
<td>138.4±7.5</td>
</tr>
<tr>
<td>Oxidative activity (optical density of test solutions) o.d. at 525 μm</td>
<td>.12±.018</td>
<td>.14±.012</td>
<td>.26±.010</td>
<td>.10±.010</td>
<td>.17±.017</td>
</tr>
<tr>
<td>Correlation coefficient† r between individual serum copper levels and oxidative activities</td>
<td>.99</td>
<td>.94</td>
<td>.93</td>
<td>.97</td>
<td>.92</td>
</tr>
</tbody>
</table>

| **Adrenalectomized Rats** |                |               |            |                                  |             |
| Number of rats           | 7              | 8             | 8          | 9                                |             |
| Weights (gm) when operated | 319.4±10.6     | 339±19.5      | 329±16.7   | 327.2±22.8                      | 330±11.4    |
| Weights (gm) when killed | 358±26.8       | 353±24.4      | 313±34.4   | 312.3±15.2                      | 355±10.9    |
| Serum copper μg/100 ml   | 114.3±11.7     | 112.6±14.1    | 193.7±13.6 | 161.5±16.6                      | 141.2±15.1  |
| Oxidative activity (optical density of test solutions) o. d. at 525 μm | .12±.017 | .13±.026 | .25±.017 | .20±.021 | .15±.025 |
| Correlation coefficient† r between individual serum copper levels and oxidative activities | 0.96 | 0.99 | 0.91 | 0.95 | 0.96 |

\[
\text{Correlation coefficient} = \frac{\sum XY - (\sum X)(\sum Y)\!n}{(\sum X^2 - (\sum X)^2\!n)(\sum Y^2 - (\sum Y)^2\!n)^\frac{1}{2}}
\]

* \[\sqrt{\frac{\sum X^2 - (\sum X)^2\!n}{n - 1}}\]
U.S.P.) in doses of 0.2 ml each was injected intramuscularly on days 0 and 4. The animals were killed on day 7, as 60% of the turpentine-treated adrenalectomized animals died on the 6th day.

3) A 1/1000 dilution of epinephrine hydrochloride was diluted to 1/10,000 with sterile 0.9% NaCl solution. Injections were given subcutaneously in doses of 15 µg at 8:00 A.M., 15 µg at 1:00 P.M. and 20 µg at 6:00 P.M. daily, including Sundays. Equivalent amounts of diluting fluid were administered to the control animals.

RESULTS

The results obtained for the various groups of animals (table 1) show a good correlation between the copper concentration and para-phenylenediamine oxidase activity of the serum. Although identical techniques were used, the oxidative activity of rat serum was much lower than that of human serum.

Sham-Operated Animals. No significant difference between the serum copper concentration and oxidase activity of sham-operated animals injected with saline or oil of sesame, respectively (table 1), was noticed. However, sham-operated rats treated with turpentine, estradiol or epinephrine showed a significant increase in the copper concentration and oxidative activity of the serum, which was especially striking after turpentine administration.

Adrenalectomized Animals. The average copper concentration and oxidase activity of serum from adrenalectomized rats treated with saline or oil of sesame, respectively, differed little, if at all, from that of similarly treated sham-operated animals. Adrenalectomized rats treated with turpentine, estradiol or epinephrine showed an increase in the copper concentration and oxidase activity of their serum, similar to that noticed in sham-operated animals treated in a like manner. This increase was slightly less after epinephrine administration.

DISCUSSION

The parallelism between the copper concentration and oxidase activity of rat serum was similar to that found in human serum, although of a different order, as mentioned above. It has been reported that there is little difference between the ceruloplasmin concentration of human and rat sera (5) but the low para-phenylenediamine oxidase values obtained with the Ravin test for rat serum as compared with human serum, using identical techniques, suggest a major difference between the two sera with respect to their ceruloplasmin content and/or phenylenediamine oxidase activity. The serum copper level of human males averages about 115 µg/100 ml, which is similar to the level found in rats. However, the serum oxidase activity of human beings approximates 0.40 U (3), which is about three times as high as that found in untreated rats. According to Gubler et al. (5), copper is present in human, rat, dog and pig plasma in at least two different forms. The one fraction reacts with sodium diethyldithiocarbamate only after removal from the protein by treatment with hydrochloric acid and corresponds to the copper α-globulin (ceruloplasmin) isolated by Holmberg and Laurell (4, 6). This fraction constitutes approximately 96, 99, 88 and 58% of the copper normally present in human, rat, dog and pig plasma, respectively. It is believed that copper α-globulin protein is mainly responsible for the reaction of paraphenylenediamine with serum in the Ravin test. These species differences may prove to be a clue to a better understanding of ceruloplasmin function.

The hypercupremic action of turpentine in the present study confirms the observations of Gubler et al. (15). The ability of the organism to raise its serum copper level under stress, therefore seems to be independent of the adrenal glands. The results are in agreement with reports of the inability of ACTH and cortisone to influence the copper concentration in turpentine-treated rats (15).

Estradiol markedly raised the serum copper levels and ppd oxidative activity of rat sera. This relationship between serum copper and female sex hormone corresponds well with the observations of various other workers (1, 11, 13, 19) and suggests a possible interrelationship between ceruloplasmin and the female sex hormones. According to our data, such interrelationship would be independent of the adrenal glands.

At present it is not clear whether the increase
in serum copper and ppd oxidase observed must be regarded as signs of tissue destruction or tissue proliferation. The fact that copper (ceruloplasmin) increased significantly during pregnancy, and that it is raised by estrogen as well as turpentine, both of which stimulate connective tissue proliferation, and the fact that it was unexpectedly high in rats with clean, dry healing wounds (data not included in tables) suggests that ceruloplasmin might be directly or indirectly associated with the proliferation and eutrophy of certain types of tissue.

The hypercupremic action of epinephrine noticed in the present study, was probably significant and independent of the adrenal cortices. This effect of epinephrine might well be an important factor in the production of slightly higher average serum copper levels and phenylenediamine oxidative activity noticed in mental patients (3, 7, 20). The stress to which a patient in an acute stage of mental illness is subjected may favor an increase in epinephrine secretion which in turn can cause a moderate rise in ceruloplasmin concentration and/or ppd oxidase activity. In the absence of such stress, the patient, although mentally ill, need not be expected to show any increase in ceruloplasmin and oxidase activity.

In the present study adrenalectomy did not effect the serum copper concentration. This observation is at variance with the hypercupremia noticed in patients with Addison’s disease (8, 14). Whether this discrepancy must be ascribed to a species difference or to the conditions responsible for the development of Addison’s disease rather than the degeneration of the adrenal glands as such, is not clear at present.

REFERENCES