STUDIES ON SERUM COPPER

I. THE COPPER CONTENT OF BLOOD SERUM IN PATIENTS WITH PSORIASIS*

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It has been established by a number of analytical and experimental observations that copper plays an essential role in the normal keratinization process of animal skin [1, 2, 3, 4].

Using histochemical techniques to study the structural development of wool fibers in sheep fed on a copper deficient diet, Marston demonstrated that the conversion of sulfhydryl groups to disulfide linkages was both delayed and diminished [3]. The total sulfur content of the wool fibers was 10–15% below normal, and the fibers were weakened, with loss of crimp. He postulated a direct catalytic role of copper in the oxidative closure of sulfhydryl residues to disulfide linkages.

Ellis et al. studied wool roots microchemically, and demonstrated partial disappearance of copper during the transformation of wool roots to mature fibers [2]. Smith and Ellis described a copper deficiency syndrome characterized by achromotrichia, alopecia, and scaly dermatosis in rabbits, and they observed clearing of the dermatosis upon dietary copper supplementation [4]. Burley and DeKock, analyzing the N-terminal amino acid residues of wool, found definite differences between the terminal groups of wool from normal and those of wool from copper deficient sheep [1].

In human subjects, blood copper levels have been assayed both under normal conditions and in a large variety of pathological states, including infectious, nutritional, metabolic, endocrine, and neoplastic disorders. Several competent reviews of the subject are available [5—12].

Only little attention, however, has been devoted to man to the copper "metabolism" in cutaneous disorders, although those characterized by aberrations in keratinization apparently would deserve such attention in great measure. In this regard, the report by Findlay and Venter is pertinent that serum copper levels are markedly elevated in pellagra, a condition usually associated with inflammation and desquamation of the skin [13]. Although absolute values were not presented, the pellagrous subjects were found to have serum levels up to double that of the normal subjects investigated.

In view of the paucity of information about blood copper levels in other scaly dermatoses, it was thought to be of interest to find out whether or not a significant difference exists between serum copper levels of psoriatic patients and those of non-psoriatic subjects. It is the object of this presentation to report on assays conducted along these lines.

METHOD

A. Methods Available

Quantitative assays of serum copper necessitate the use of a highly sensitive procedure because of the very low concentrations present. The diethyl dithiocarbamate and dithizone reagents widely utilized in previous colorimetric assays are not adequately sensitive. They are, moreover, not sufficiently selective, as they react with other trace metals as well, thus producing other colored combinations which call for intricate extraction procedures [14].

In the present study, therefore, the serum copper levels were determined by the spectrophotometric procedure of Peterson and Bollier [14]. This method utilizes the tendency of bis-cyclohexanone-oxalylidihydrazone to combine with copper and hereby to form a product of intensely blue coloration. The sensitivity of this reagent has been found superior to that of the previously used substances, yet no disturbing color reaction was observed with any of the other electrolytes commonly encountered in biological materials.

B. Further Elaboration of Procedure

Reagent Stability—In our efforts to ascertain the stability of the solutions employed in order to carry out the reactions under strictly comparable conditions, it was found that the standard reference solutions of copper had undergone decomposition after two weeks of storage at room tem-
perature, and somewhat later at refrigerator temperatures.

When the reagent solution was added to aqueous serial dilutions of a stock solution of cupric sulfate which had been prepared two or more weeks before, the spectrophotometric transmission readings obtained were too high. The corresponding Cu readings amounted to only 90% to 98% of the Cu readings obtained with the freshly prepared copper solution and its dilutions.

It became evident, therefore, that fresh standard solutions of cupric sulfate must be prepared at intervals of ten days to [not more than] two weeks, and stored under refrigeration. Observing these new precautions, two investigators, in independent “blind” determinations of the copper concentration in prepared solutions or in different specimens of blood serum, obtained values which differed by 5% or less from each other.

Specimen Stability—All serum specimens were obtained by separation from freshly clotted blood. Thereafter, the copper values remained equal up to two weeks for any given specimen stored at refrigerator temperatures.

SUBJECTS

Serum copper values were assayed in 26 healthy subjects, 14 white males, 23 to 45 years of age—and in 12 white females, 23 to 55 years of age.

**TABLE I**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sex</th>
<th>Number of Subjects</th>
<th>Mean Serum Copper in MCG/ML</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Control Subjects</td>
<td>M</td>
<td>14</td>
<td>1.58</td>
<td>1.00-2.73</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>Psoriatic subjects</td>
<td>M</td>
<td>20</td>
<td>2.02</td>
<td>0.63-2.96</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9</td>
<td>2.02</td>
<td></td>
</tr>
</tbody>
</table>

*We wish to thank Mrs. Lee F. Herreras, Department of Medical Statistics, New York University Medical Center, for advising us in the statistical analysis and presentation of the results obtained.

In addition, the serum analyses were performed in 29 outpatients with typical psoriasis. Twenty of these patients were white males, 23 to 69 years old. The remaining nine patients were white females, 13 to 60 years old. The duration, extent, and activity of the eruptions varied widely among the subjects.

At the time of the first blood collection, some of the patients had been receiving external treatment with medications other than those containing steroid, but none had received any systemic-, radiation-, or heliotherapy within the preceding three months.

RESULTS

The findings obtained in our healthy control subjects and in the psoriatic patients are listed on Table I. The mean value of serum copper found in healthy subjects averaged below the value found in the subjects affected by psoriasis. This difference has been significant statistically for the males \[0.01 < P < 0.02\], but failed to reach significance in the smaller groups of females \[0.2 < P < 0.3\]. Apparently, there was no sex difference between our values obtained in either psoriatic or healthy subjects.

When the subdivision according to sex is disregarded, the difference between the results obtained in all our psoriatics and those obtained in all of the control subjects would actually be significant \[P < 0.01\]. A considerable overlap, however, is apparent in the serum copper levels of normals and psoriatics, with some of the lowest values found in psoriatic subjects, and some of the highest values in healthy subjects [see graph].

DISCUSSION

Our finding of elevated serum copper levels in psoriatics requires further investigation before definite conclusions can be drawn.

It will be of crucial importance to exclude all subjects from the investigation who show peculiarities or pathological conditions known to be
possibly associated with elevation or depression of blood copper. Elevation has been observed in a variety of states, including the last trimester of pregnancy, hyperthyroidism, Addison’s disease, certain hypochromic anemias, pernicious anemia, aplastic anemia, leukemias, lymphomas, carcinoma, various subacute and chronic infections, and certain hepatic diseases [5, 6, 7, 8, 10, 11]. Inflammatory and febrile states in general apparently tend to show some elevation in blood copper, and this tendency might have a bearing on our present observation.

Decrease in blood copper has been recorded in some cases of Wilson’s disease, severe nephritis with hypoalbuminemia, acute leukemias treated with ACTH, and hypothyroidism [8, 9].

To the best of our knowledge, none of the patients and control subjects included in our present study was afflicted by any of those conditions.

Whereas Lahey et al. did not observe any considerable diurnal, daily, or weekly fluctuations in the plasma copper level, or any such fluctuations with regard to intake of food or to menstruation, these authors did note significantly higher plasma copper levels in females than in males. This sex difference was not apparent in the present group [7, 8] either among normals or psoriatics. Should the difference by chance have been obscured in our relatively small number of [healthy] persons, the predominance of males over females in the psoriatic group would, with some probability, have resulted in a lower serum copper among the psoriatics than among the non-psoriatics, whereas the reverse was actually found.

To our knowledge, no aberration in serum copper levels has been reported thus far in psoriasis. Whether the finding observed by us is due to inability of the epidermal cells to utilize copper in the formation of keratin, or whether the excess copper is derived from some other source, remains to be studied. Of relevance to the question is the report of MacCardle et al. of elevated tissue copper levels in psoriatic skin on spectrographic analysis [15]. It would be of interest to extend such tissue analyses to other scaly dermatoses.

It furthermore would be of interest to find out to what extent hypercupremia is encountered also in other dermatoses associated with para-keratosis. It would appear to be more likely that the elevation in serum copper somehow is secondary to the imperfect keratinization, rather than a causative factor.


DISCUSSION

DR. PETER FLESCH, Philadelphia, Pa.: Although with Dr. Rothman we are among the people who developed the idea of the role of copper in keratinization, I would like to emphasize that this is proved in the case of wool only. Copper may have nothing to do with epidermal keratinization. There is also an overlapping of the copper values and I wonder how valid the statistical analyses are.

DR. GEORGE LIPKIN, (in closing): I would like to thank Dr. Flesch for his comments. We are aware of the pitfalls inherent in transferring to human epidermis findings obtained in animal species, particularly with hair keratins. Nevertheless, we feel the subject merits investigation.

Our statistical methods were standard ones, and adequate for the study.

Subsequent to submission of this paper some additional work has been done, which was omitted for the sake of brevity. First, in cooperation with Dr. Bearn of the Rockefeller Institute, the serum ceruloplasmin levels were determined in six psoriatic patients. Mild elevation was present in four of the six, but the number of subjects was too small for statistical significance. Since about ninety-five percent of serum copper is bound to ceruloplasmin, one might expect any tendency toward serum copper elevation to be reflected in this fraction.

Tissue copper was also determined in involved skin of twelve psoriatics, and in contradiction to an earlier report of elevation (Mac Cardle, R. C., Engman, M. F., Jr., and Engman, M. F., Arch. Dermat. and Syph. 44: 429 (Sept. 1941)), was within the normal range. One cannot, however, rule out the presence of some more subtle enzymatic abnormality in copper metabolism, despite absence of any gross quantitative alteration.


Whether these findings have any relevance to the problem of psoriasis remains to be determined.