Non-Dialyzable Manganese, Copper and Sodium in Human Bile¹,²

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The highest concentration of copper and manganese of all body organs has been found in the liver (1). The major pathway of excretion of these metals is via the stool, with small quantities being excreted in the urine. It is therefore assumed that the liver is the main pathway of excretion of these materials. Gallbladder bile has been reported to contain from 24-1070 μG Cu/100 ml (2, 3). Bile collected from the duodenum of normal subjects was reported to contain 6-20 μg Cu/100 ml (4, 5). Gallbladder bile has been reported to contain about 1 μg Mn/100 ml, as non-dialyzable manganese, while pancreatic juice contained nearly twice this value (6). The excretion of Mn is almost totally via the gastrointestinal tract (7, 8), very little being eliminated in the urine (7, 9).

The purpose of this report is to present copper, manganese and sodium concentrations, based on neutron activation analysis of dialyzed bile, obtained from gallbladder of subjects without history of biliary disorders and surgical subjects for cholecystectomy and from T-tube drainage.

METHODS AND MATERIAL

Between 5 and 10 ml of bile was removed from the gallbladder of 39 male subjects during postmortem examination. Stainless steel needles and plastic syringes were used for these collections. These samples were transferred to properly cleaned test tubes and centrifuged for 15 minutes at 3000 rpm. One ml aliquots of supernatent fluid were used for dialysis and subsequent analysis for copper, manganese and sodium by the procedure described previously (10). The remaining portions were stored in a deep freeze, −9°C, until used for other tests.

¹Partially supported by U.S. Department of Health, Education and Welfare, National Institute of Health Grant No. RG-9045.
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About 4 ml of bile was also obtained from eight surgical subjects undergoing cholecystectomy. These samples were collected and processed in the same manner as those described above.

Variable amounts of bile were collected, via T-tube, from six post-surgical patients. Three of these subjects were included in the group of eight surgical patients, prior to cholecystectomy. Drainage material was collected in plastic bags. These samples were also processed in the manner described above.

Total protein determinations were carried out on all samples collected, using a modified Biuret method (11).

RESULTS

Collected bile appeared to be heterogenous in character. Its color ranged from light brown, red brown, black brown to black green. Its viscosity ranged from watery to stringy mucous consistency. Analysis of centrifuged and non-centrifuged, random samples, indicated differences in concentrations of sodium, copper and manganese. For example, a non-centrifuged mixture produced the following non-dialyzable levels: Cu-388 µg/100 ml; Mn-3.2 µg/100 ml; Na-42 µg/100. The supernatent portion of the same centrifuged sampled yielded the following levels: Cu-309 µg/100 ml; Mn-2.1 µg/100 ml; Na-34 µg/100 ml. All results presented in this study, are based on analysis of supernatent portions of centrifuged bile.

The non-biliary disease group of subjects, consisted of patients who expired, due to the following causes: Twenty-two subjects with heart disease; five with carcinoma; and the remainder with miscellaneous diseases. There were no significant differences in results obtained among the group indicated. There were three subjects in the non-biliary disease group, who were not included in the above group of 36. The data of these subjects was singled out and is presented in Table II. Post-mortem pathological examination of the subject with carcinoma and heart disease revealed that both these subjects had bronchopneumonia, fibrous pleuritis and pulmonary fibrosis.

DISCUSSION

The non-dialyzable concentrations of bile manganese represent the major contribution of the present study. Comparison of levels obtained with work of other investigators cannot be made at this time, since no reports were found in the literature. Increased levels of manganese, have been noted previously in the serum of patients with myocardial infarction (10) and in the cerebrospinal fluid of subjects with the presence of necrotic lesions (12). The elevated manganese levels in the bile of the three subjects listed in Table II, can be correlated with elevated biliary protein concentrations, which are considerably greater than the average values noted in the bile, of subjects with biliary and non-biliary diseases. (Table I). The protein levels in these two groups of subjects are considerably greater than the normal bile protein levels reported by Altman and Dittmer (13), who give values of 315 to 539 mgs/100 ml for gallbladder bile and 273 mgs/100 ml for liver bile.
Judd and Dry (2) reported copper concentration in human bile, ranging from 63 to 1070 μg/100 ml. Cartwright and Wintrobe (3) reported copper levels in human gallbladder bile, ranging from 24 to 538 micrograms per 100 ml. The latter report also indicated that all the copper in the bile is non-dialyzable. The results obtained in the present study represent the non-dialyzable levels of copper, which in general agree with the values cited by the above mentioned investigators. The non-dialyzable copper, in the bile of subjects without the history of biliary disorders, averaged 544 ± 329 μg/100 ml (Table I), while the copper in the bile of subjects with the biliary disorders was somewhat less in concentration, 341 ± 316 μg/100 ml. The copper levels noted in the T-tube collected bile were considerably lower in concentration, 37 ± 21 μg/100 ml, agreeing with the levels reported previously by Cartwright and Wintrobe (3).

### Table I

**STUDY OF HUMAN BILE IN SUBJECTS WITH AND WITHOUT BILIARY DISORDERS**

<table>
<thead>
<tr>
<th>Patient category</th>
<th>No. of subjects</th>
<th>Non-dialyzable Protein (grams/100 ml)</th>
<th>Copper (μg/100 ml)</th>
<th>Manganese (μg/100 ml)</th>
<th>Sodium (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-biliary diseases</td>
<td>36</td>
<td>1.8 ± 1.0</td>
<td>554 ± 329</td>
<td>1.1 ± 0.6</td>
<td>5,104 ± 2,113</td>
</tr>
<tr>
<td>Biliary diseases</td>
<td>8</td>
<td>1.4 ± 0.9</td>
<td>341 ± 316</td>
<td>2.1 ± 2.1</td>
<td>1,689 ± 1,530</td>
</tr>
<tr>
<td>T-tube drain</td>
<td>6</td>
<td>0.7 ± 0.2</td>
<td>37 ± 21</td>
<td>2.1 ± 2.9</td>
<td>54 ± 39</td>
</tr>
</tbody>
</table>

The indicated ± signs represent standard deviations.

### Table II

**COMPARISON OF GALLBLADDER BILE DATA IN THREE SUBJECTS WITH INCREASED MANGANESE CONCENTRATION**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Protein (grams/100 ml)</th>
<th>Copper (μg/100 ml)</th>
<th>Manganese (μg/100 ml)</th>
<th>Sodium (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpura-echymosis</td>
<td>1.6</td>
<td>47</td>
<td>7.7</td>
<td>89</td>
</tr>
<tr>
<td>Bronchogenic carcinoma</td>
<td>6.5</td>
<td>612</td>
<td>3.5</td>
<td>913</td>
</tr>
<tr>
<td>Arteriosclerotic heart disease</td>
<td>3.2</td>
<td>151</td>
<td>6.6</td>
<td>328</td>
</tr>
</tbody>
</table>
Sodium content of human bile has been reported to range between 300 and 380 mgs/100 ml. The non-dialyzable sodium levels of bile in the present study (Table 1) represent but a small portion of the total sodium. Statistically significant differences were noted in the sodium levels of bile among the three groups of bile collected. The non-dialyzable sodium concentration was greatest in the bile of subjects without biliary disease, less in subjects with biliary disorders and least in the T-tube collected bile. The agonal effect upon sodium concentration in the post mortem group can not be assessed.

Generally speaking, the protein, copper and sodium levels appeared to be greater in concentration, in the bile of subjects having no history of biliary disorders, than in the subjects with biliary disease. An explanation for this observation, may be in the inability of the diseased gallbladder to concentrate the components mentioned, or in the inability of the liver to supply amounts of these substances in concentrations normally present.

SUMMARY

Gallbladder bile was collected post-mortem from 36 adult male subjects, who were without history of biliary disorders and from eight patients undergoing cholecystectomy. T-tube bile collections were also made on six post-cholecystectomy patients. Collected samples were analyzed for protein and non-dialyzable content of copper, manganese and sodium.

The results indicated that greater concentrations of protein (1.8 ± 1.0 grams/100 ml), non-dialyzable copper (554 ± 329 µg/100 ml) and non-dialyzable sodium (5,104 ± 2,113 µg/100 ml) were present in the gallbladder bile of subjects without history of biliary disorders, than in subjects with biliary disorders (protein 1.4 ± 0.9 grams/100 ml; copper 341 ± 316 µg/100 ml; and sodium 1,689 ± 1,530 µg/100 ml).

The non-dialyzable manganese level in the non-biliary disease group of 36 subjects averaged 1.1 ± 0.6 µg/100 ml, while in the biliary disease group of eight subjects the manganese level was 2.1 ± 2.1 µg/100 ml.

ACKNOWLEDGMENT

The authors are gratefuly indebted to Stephen J. Sontag of Radioisotope Research, Veterans Administration Hospital, Hines, Illinois, for his untiring efforts in obtaining the material used in this study and for his sincere and genuine interest and cooperation.

REFERENCES


STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

(In accordance with Act of October 23, 1962: Section 4369, Title 39, United States Code.)

Statement filed October 1, 1967 for the Journal of Nuclear Medicine, which is published monthly, (January, February, March, April, May, June, July, August, September, October, November and December). Headquarters of publication and business office located at 333 North Michigan Avenue, Chicago, Cook County, Illinois 60601. Publisher is Samuel N. Turiel & Associates, Inc. located at 333 North Michigan Avenue, Chicago, Illinois 60601. The Editor is Dr. George E. Thoma, St. Louis University Medical Center, 1402 S. Grand Boulevard, St. Louis, Missouri 63104. The Managing Editor is Samuel N. Turiel, 333 North Michigan Avenue, Chicago, Illinois 60601. The Journal of Nuclear Medicine is the official publication of the Society of Nuclear Medicine Inc. located at 333 North Michigan Avenue, Chicago, Illinois 60601. There are no known bondholders, mortgages and other security holders owning or holding 1 per cent or more of total amount of bonds, mortgages or other securities. The average number of copies printed during the preceding twelve months is 4850 of which 4850 were mailed to members of the Society of Nuclear Medicine and paid subscribers. There were no sales through agent or news dealers. In addition 200 copies were distributed without charge to advertising agencies and advertisers and those seeking sample copies. This provides a total distribution of 4850 copies on an average basis. For the October, 1967 issue of the Journal of Nuclear Medicine 4899 were printed of which 4899 copies were mailed to members of the Society and paid subscribers, 211 were distributed without charge providing a total distribution of 4899.

1 Samuel N. Turiel, Managing Editor of the Journal of Nuclear Medicine, certify that the statements made above are correct and complete.