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Manganese and Copper Levels in Human Urine[1,2,3]

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The application of neutron activation analysis for detection of trace elements in various fields of science has been made by many investigators. This laboratory has reported on Mn and Cu levels in serum (1), cerebrospinal fluid (2), saliva (3), and pancreatic fluids (4), and is currently engaged in further exploration of biological material by means of this technique.

Relatively little is known of Mn levels in urine, or of its character of occurrence in normal subjects and subjects with a variety of diseases. Little is also known of the chemical character of Cu occurring normally in the urine. This is more adequately covered in the discussion.

It seemed appropriate, therefore, to report at this time the results of more recent studies which were based on a large number of urine specimens obtained from normal male subjects and subjects hospitalized with a variety of diseases and disorders. Manganese and Cu levels in the urines studied will be presented, together with some data on the character of non-dialyzable metal moiety occurring in normal subjects.

METHODS AND MATERIAL

Sixty-four daily urine collections were obtained from 16 apparently healthy male subjects, ranging in age from five through 47 years. Each of the subjects contributed four 24-hour urine specimens. No attempt was made to control the diet or restrict the daily activities of these individuals. None of the subjects was on medication.

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[2]From the Radioisotope Research Service, Veterans Administration Hospital, Hines, Illinois, and Stritch School of Medicine, Loyola University, Chicago.

[3]Presented at the 11th Annual Meeting of the Society of Nuclear Medicine, Berkeley, California, June 20, 1964.

Forty-five daily urines were also obtained from 14 hospitalized adult male subjects. Diagnosis of these patients included myocardial infarction, cirrhosis of the liver, carcinoma of the bladder, Wilson's disease, and schizophrenia with severe depression.

All urines were collected in four liter polyethylene bottles and cleaned according to the procedure described previously (1). These urines were preserved with thymol crystals (free of Mn, Cu and Na, as based on analysis of the gamma-ray spectrum after sample irradiation with thermal neutrons), and stored in a refrigerator (9°C). These urines were examined for acidity (all urines ranged between pH 5.5 and pH 6.5), albumin (all urines were negative to "heat and acetic acid test"), and reducing substances (all urines were negative to qualitative Benedict Reagent) according to standard procedures employed in a clinical laboratory (5). Creatinine determinations (6) were performed on all collected urines in order to ascertain the validity of complete daily collections.

TOTAL MANGANESE

Manganese was precipitated as MnO_2 by boiling urine with concentrated HNO_3 containing $KClO_3$ according to the procedure of Bowen and Cawse (7). The Mn was then quantitated by neutron activation analysis of the MnO_2.

Attempts to quantitate Mn in urines of normal subjects by means of atomic absorption technique (Spectrophotometer, Perkin-Elmer Model 303) were unsuccessful. A satisfactory response could not be obtained when using urine concentrated 10-fold. Reproducible results were not obtained in our laboratory by the spectrophotometric procedure of Bolon *et al* (8). In addition, solvent extraction subsequent to chelation of Mn with acetylacetone according to Devoe (9), and followed by neutron activation analysis was not successful since good recoveries were not obtained.

NON-DIALYZABLE MANGANESE

One ml aliquots of each daily urine collected were dialyzed and analyzed by the neutron activation technique described previously (1). One ml aliquots of urine concentrates were also analyzed by the same technique. These concentrates were obtained as follows: Ten percent of each daily urine collection was pooled and 100 ml aliquots of such pools were lyophilized. The dried residue was reconstituted in buffer. One ml portions of the final concentrates represented between 7.4 and 8.2 ml of original urine pool.

Correction was made for buffer contribution of Mn to the non-dialyzable level obtained by means of "reversed dialysis" technique. Sixty-five ml aliquots of buffer were irradiated in quart vessels, together with a similar volume of buffer containing Mn standard. Dialysis procedure (1) was carried out using this irradiated buffer, against 1.0 ml aliquot of each urine concentrate. Immediately following dialysis, the sample aliquot was diluted to 10 ml volume, examined for its gamma-ray spectrum, and assayed for the Mn present. Factors of such an assay included the counts obtained for the urine aliquot (1.0 ml of urine plus 9.0 ml of non-irradiated buffer), sum of the counts obtained for six 10.0 ml

aliquots of irradiated buffer, and the Mn concentration in six 10 ml aliquots of irradiated buffer. The quantity of Mn was then expressed in terms of concentration per 24 hour urine volume, and represents the actual amount of Mn contributed by the buffer. The corrected urine Mn concentration was obtained by subtracting the buffer contribution from the non-dialyzable level obtained after dialysis and analysis of the concentrate.

Reproducibility of Mn standard solutions analysis, in concentrations of 0.8 ug/liter, 2.5 ug/liter and 16.6 ug/liter, were ± 44 per cent, ± 15 per cent, and ± 5 per cent respectively. These concentrations represent levels observed in urine, cerebrospinal fluid (and saliva), and serum. The lack of reproducibility of standards having similar concentration as non-concentrated urine, points directly to the need for concentration of urine which is to be analyzed for Mn. Reproducibility of Mn determinations of urine concentrates was estimated to be ± 9.6 per cent. In all instances ± represents standard deviation.

TOTAL COPPER

One hundred ml aliquots of each normal urine were digested with nitric acid, perchloric acid and sulfuric acid, and analyzed for Cu by means of Na-diethyldithiocarbamate according to the procedure described by Eden and Green (10).

NON-DIALYZABLE COPPER

Copper determinations were carried out concurrently with the assay for Mn using the neutron activation technique. Buffer contribution to the non-dialyzable urine Cu were also carried out in a similar manner as for Mn.

RESULTS IN NORMAL SUBJECTS

TOTAL MANGANESE

No significant values for urine Mn can be cited on the basis of chemical estimations. The methods employed did not seem to possess the required sensitivity, recovery, or both.

NON-DIALYZABLE MANGANESE

Typical gamma-ray spectrum of dialyzed non-concentrated urine of normal subject is presented in Fig. 1. Relatively low levels of ^{56}Mn in 1.0 ml urine aliquots necessitated an 800 second counting. Many of the urines however required only 400 second counting. The difference in counting time is attributed to differences in daily urine volumes which ranged from 460 ml to 2330 ml per day in the group of subjects studied.

Six pools of a normal human urine were concentrated by lyophilization. The non-dialyzable Mn levels were established by dialysis and neutron activation. The buffer contribution was estimated by the "reversed dialysis" described

under METHODS AND MATERIALS. The corrected levels were obtained by the difference between the non-corrected concentration and the buffer contribution. Summary of these studies are presented in Table I, and indicate clearly the extent of these contributions at different levels of Mn present in the urine. Extent of contamination by other components were found to be insignificant (1,2).

It appears that considerable corrections must be made when urine Mn concentrations are of a very low magnitude. Based on the above data the following corrections were derived: When the non-dialyzable Mn level in the urine is 0.3 ug per day one must subtract 0.18 ug since 61 per cent (average of 74.42 per cent and 50.00 per cent) of 0.3 ug is attributable to the Mn from the buffer. Similarly then the non-dialyzable level between 0.5 and 1.0 ug per day must have subtracted 0.15 ug (29.48 per cent of 0.51 ug) and 0.26 ug (27.08 per cent of 0.96 ug), respectively. When the Mn level increases to about 2.6 ug per day, the amount to be subtracted becomes considerably less, 0.25 ug (11 per cent of 2.35 ug).

Using the above factors, appropriate corrections were made on all Mn values obtained for urines of normal subjects. This is justified on the basis that these correction factors were established for the same urines (actually 59 urines out of 64 were used for such correction factors). The corrected Mn levels are presented in Table II together with the age of each subject, surface body area (calculated according to Dubois and Dubois), urine volume, and creatinine concentrations.

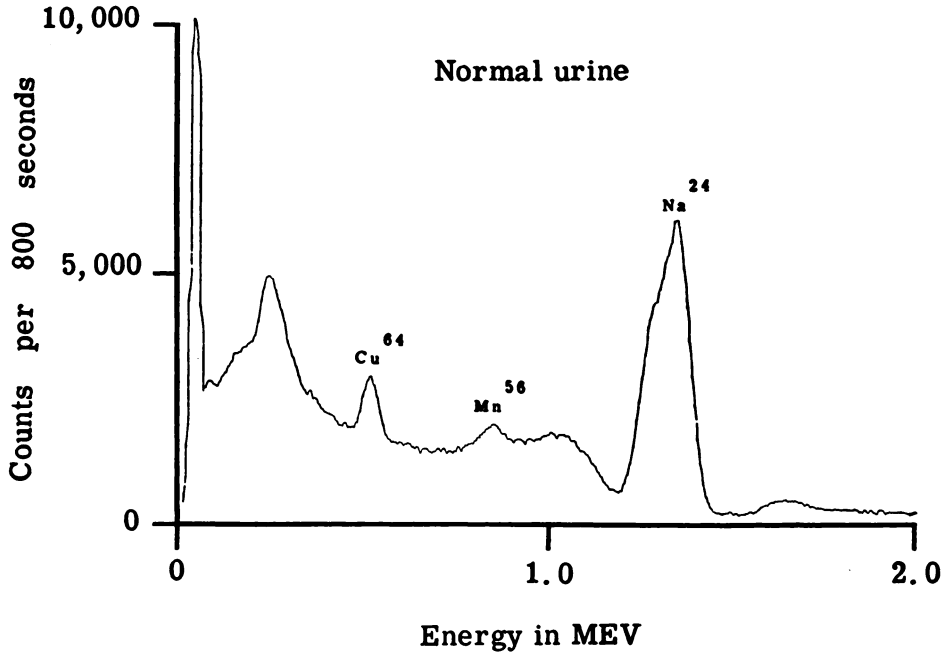

Fig. 1. Gamma-ray spectrum of dialyzed 1.0 ml urine aliquot obtained from a normal subject, approximately 1-hour after a 30-minute irradiation.

TABLE I

AVERAGE NON-DIALYZABLE Mn CONCENTRATIONS IN POOLED NORMAL HUMAN URINE BEFORE AND AFTER BUFFER CONTRIBUTION CORRECTION.

No. specimens per pool	*Micrograms Per Daily Urine*						*Average*
	12	*4*	*7*	*12*	*12*	*12*	
Non-corrected level	0.29	0.32	0.51	0.96	2.09	2.60	1.29
Buffer contribution	0.21	0.16	0.15	0.26	0.16	0.37	0.23
Corrected level	0.08	0.16	0.36	0.70	1.93	2.23	1.06
Percent correction	72.42	50.00	29.48	27.08	7.66	14.23	17.83

The corrected Mn levels were extrapolated to the basis of average normal body surface area, 1.73 m^2. No relationship between the Mn concentration and the age of the subjects was observed.

TABLE II

AVERAGE NON-DIALYZABLE Mn LEVELS OF FOUR 24-HOUR URINE SPECIMEN OBTAINED FROM EACH OF 16 APPARENTLY HEALTHY MALE SUBJECTS

Subject	*Age*	*Surface body area, m^2*	*24-hr Volume ml*	*Creatinine gms/vol*	*Mn ± std. dev μg/day*
1	5	0.90	645	0.43	0.42 ± 0.28
2	10	1.32	692	0.83	0.38 ± 0.24
3	14	1.94	992	1.76	1.09 ± 0.38
4	20	1.53	760	1.04	0.36 ± 0.21
5	22	1.63	1300	1.10	0.09 ± 0.04
6	26	1.88	590	0.73	0.12 ± 0.01
7	27	1.99	1482	1.06	1.32 ± 0.81
8	32	1.80	1188	1.10	1.18 ± 0.56
9	37	2.04	1280	0.98	0.53 ± 0.23
10	37	2.02	1872	1.12	1.10 ± 0.43
11	38	1.88	1222	1.42	0.94 ± 0.18
12	38	1.98	1206	1.14	1.01 ± 0.27
13	40	2.05	1252	0.99	1.25 ± 0.59
14	41	1.81	1739	0.92	1.26 ± 0.50
15	46	1.81	1014	1.06	0.52 ± 0.09
16	47	1.90	2240	0.67	1.62 ± 0.68
Average		1.78	1340	1.10	0.82
Std. Dev.		±0.29	±625	±0.41	±0.34

Manganese concentrations in each of the 64 daily urines studied were plotted according to their frequency of occurrence, Fig. 2.

TOTAL COPPER

The average total Cu concentration in 64 urines of normal subjects studied was 50.37 ± 11.14 ug per day.

One additional 24-hour urine specimen was collected from each of six of the above subjects for the purpose of establishing the extent of Cu contribution from urine sediment components. Analysis were made for total Cu, using the method of Eden and Green (10). Total concentration in the freshly collected, well mixed urine, averaged 48.82 ± 21.51 ug per day, while the total Cu concentration of the supernatent portion of these urines following centrifugation (30 minutes at 3000 rpm) averaged 45.72 ± 16.00 ug per day. Therefore, the sediment components of normal urine represented about 6.7 per cent of the total Cu present.

NON-DIALYZABLE COPPER

Concentrated urine pools used for establishing the buffer contribution to Mn levels were also used for establising similar contribution to the Cu levels obtained. The method employed was similar to that used for Mn. Summary of these studies are presented in Table III.

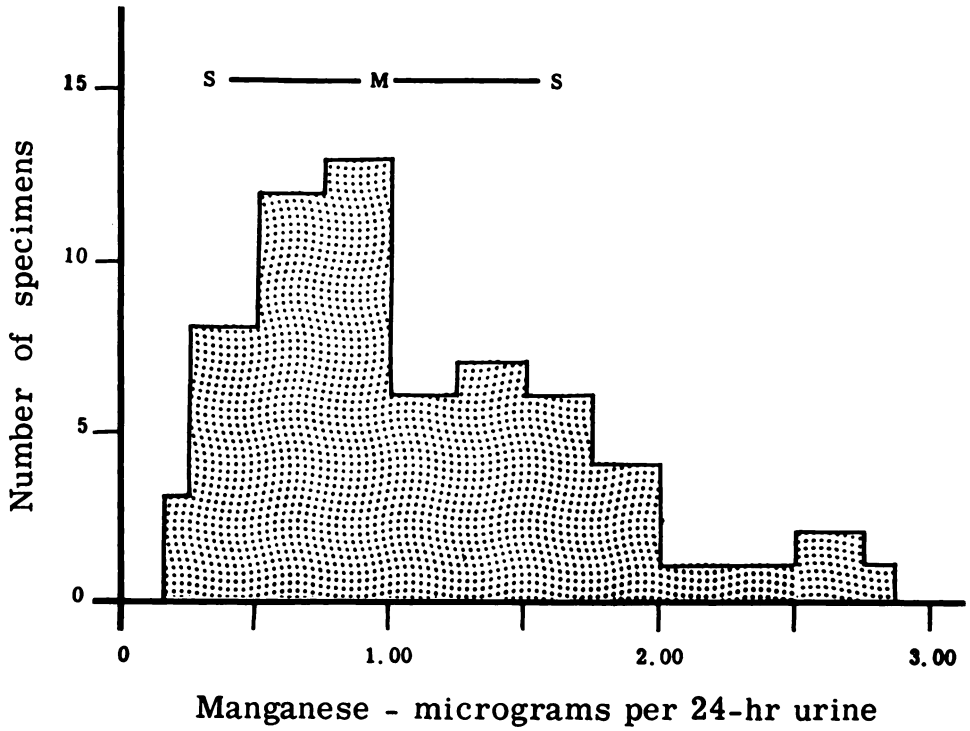

Fig. 2. Frequency distribution of the non-dialyzable Mn found in normal urine. M is the mean and S is Std. dev.

TABLE III

AVERAGE NON-DIALYZABLE CU CONCENTRATIONS IN POOLED NORMAL HUMAN URINE BEFORE AND AFTER BUFFER CONTRIBUTION

number in each pool	*Micrograms Per Daily Urine*						*Average*
	12	*7*	*12*	*12*	*4*	*12*	
Non-corrected level	27.27	42.44	43.79	50.00	59.26	67.59	47.42
Buffer contribution	5.12	9.26	15.23	7.73	6.70	8.52	8.99
Corrected level	22.15	33.18	28.56	42.27	52.56	59.07	38.43
Percent correction	18.78	21.82	34.68	15.46	11.31	12.61	18.96

It appears that no relationship exists between the non-corrected Cu levels and the percent correction required. An average correction factor of 19 per cent was used, therefore, to correct the urine non-dialyzable Cu levels for Cu derived from the buffer during the dialysis. The corrected nondialyzable Cu levels in the urines of normal subjects are presented in Table IV, together with the total.

TABLE IV

AVERAGE TOTAL CU AND NON-DIALYZABLE CU LEVELS OF FOUR 24-HOUR URINE SPECIMENS WHICH WERE OBTAINED FROM 16 APPARENTLY HEALTHY MALE SUBJECTS

Subject	*Micrograms Per Day*	
	Total ± std. dev.	*Non-dialyzable std. dev.*
1	23.87 ± 5.47	31.72 ± 8.37
2	34.66 ± 4.24	34.41 ± 13.01
3	42.25 ± 6.92	33.55 ± 6.66
4	27.63 ± 4.58	33.53 ± 10.49
5	39.78 ± 13.78	47.58 ± 13.29
7	45.60 ± 14.56	52.81 ± 5.33
8	41.31 ± 9.59	37.45 ± 11.32
9	69.55 ± 3.74	65.77 ± 8.46
10	106.56 ± 29.78	77.22 ± 27.83
11	40.89 ± 4.69	36.88 ± 8.50
12	55.67 ± 8.48	59.40 ± 8.24
13	56.84 ± 23.51	41.31 ± 6.60
14	58.69 ± 17.23	53.55 ± 5.14
15	44.93 ± 12.12	18.89 ± 5.92
16	67.47 ± 8.42	65.65 ± 6.73
Average	50.37 ± 11.14	45.98 ± 9.72

Cu levels. The average non-dialyzable Cu concentration amounts to 91.28 per-cent of the total Cu estimated in these urines.

Concentrations of Cu in each of the 64 daily urines studied were plotted according to their frequency of occurrence, Fig. 3.

HOSPITALIZED ADULT SUBJECTS

Forty-five daily urines obtained from 14 hospitalized adult subjects were analyzed for non-dialyzable levels of Mn and Cu. No correction was made for the buffer contribution to the levels obtained for Cu or Mn. The data presented in Table V includes the non-corrected concentrations for Cu and Mn in urines of normal subjects, so that a comparison could be made between the levels of apparently normal subjects and those hospitalized.

DISCUSSION

Although excretion of Cu is primarily via the gastrointestinal tract (11-14), relatively small amounts, 20-80 ug per day, are excreted in the urine of normal subjects (13-17). It is estimated that this quantity excreted in the urine represents about two per cent of the total Cu ingested daily. The Cu in the feces has also been reported to be equivalent to the daily intake of Cu in the diet (12-14). Variations in urine concentrations from day to day have been reported to be quite significant (18). The data obtained in this study indicate that these varia-

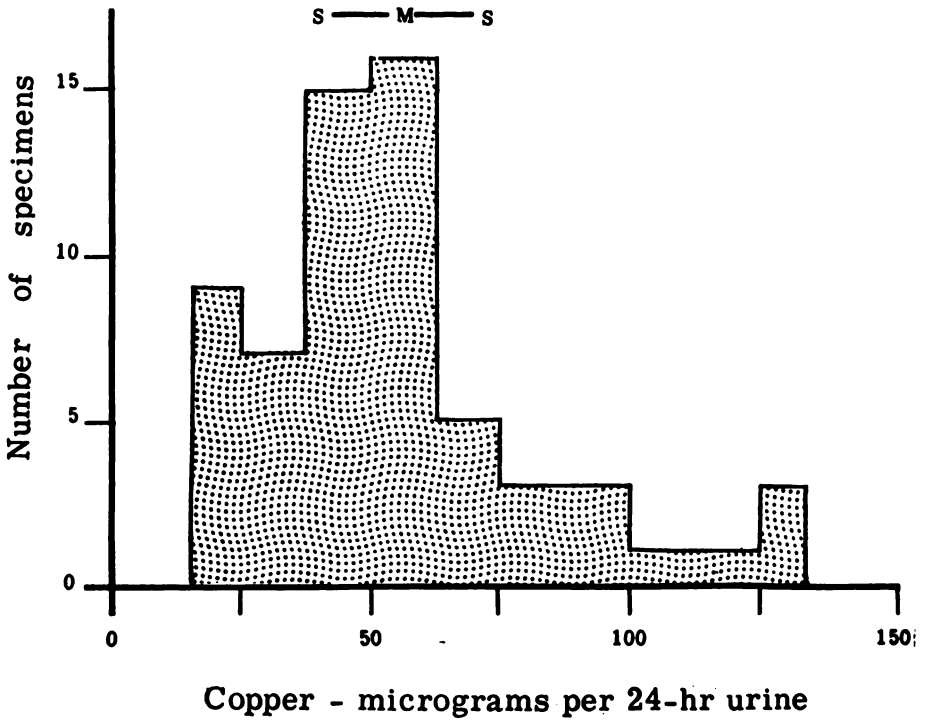

Fig. 3. Frequency distribution of the non-dialyzable Cu found in normal urine. M is the mean and S is Std. dev.

tions may be within ± 20 percent of the mean obtained in each group of urines from the same subject. It is possible that this variation may be due to variations of Cu content in daily food.

Urine excretion of Cu, 50.4 ± 11.1 ug per day, observed in this study of normal subjects, represents a mean and a range which are in excellent agreement with the normal average and range reported by other investigators (13-16).

Average urine nondialyzable Cu, 46.0 ± 9.7 per day, based on analysis of these same urines is also in conformity with the total Cu values cited. The close similarity in levels obtained for total Cu, and for non-dialyzable Cu, strongly suggest that the Cu in the urine is present in a bound form. Sediment components represent about six per cent of the total Cu present.

It is known that plasma proteins have an affinity for binding Cu (19, 20), which in plasma is nondialyzable (21, 22), and has been shown to be 90 to 100 percent protein bound (21-25). It is also known that small quantities of plasma proteins filter through the glomeruli and normally appear in the urine. Other urinary proteins may have their source in the genito-urinary tract. The daily excretion of protein in the urine of normal subjects is reported to be 193.8 ± 53.0 mg per 24-hour urine volume (26). Two thirds of these proteins represent globulins and the remainder consist of albumin and mucoproteins. It is not unreasonable, therefore, to assume that the Cu observed in the urine of normal subjects is bound completely or in part to these proteins.

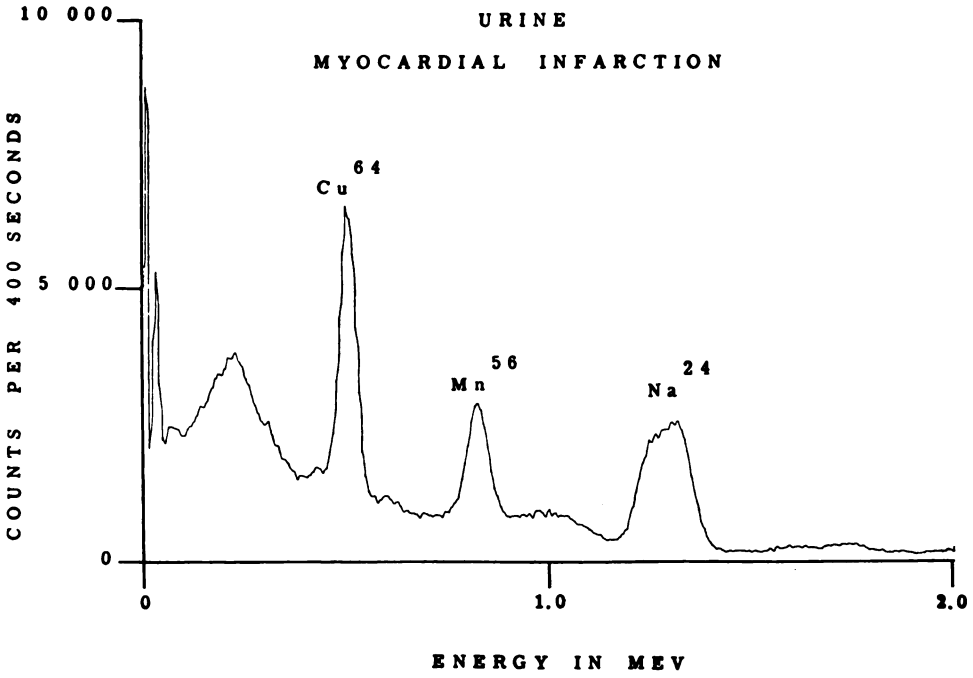

Fig. 4. Gamma-ray spectrum of dialyzed urine obtained from a patient following an acute myocardial infarction, approximately one hour after a 30 minute irradiation.

The exact biochemical nature of copper-containing substances in the urine is not known. Several origins may be postulated: They may consist of the one or more consitutents of plasma; i.e., Cu ion, organic complex with enzyme (s), or protein complexed Cu as exemplified by ceruloplasmin. No information is available to indicate a mode of transfer into the urine, whether by glomerular filtration or tubular excretion. If transferred into the urine as a Cu ion or small organic complex, it may subsequently bind to a large molecule in the urine. Another possible site or origin may be physiological turnover of the renal tubular epithelium cells which may break down and release the bound Cu into the urine. If due to the latter, a clue may be provided as to the rate of renal tubular cell turnover.

Considerably less information is available on the levels of Mn excretion or the nature of its occurrence in biological fluids, particularly the urine. In 1926 McCrackan and Passamaneck (27) reported Mn concentration in one urine to be less than 20 μg per liter. In 1940 Kehoe et al (12) reported the urine Mn concentration to be less than 10 μg per liter of urine. These authors also stated that the daily output of Mn in the feces is "practically" equivalent to the daily intake of Mn in the diet. Approximately 0.3 per cent of the ingested Mn was excreted in the urine. Cotzias, Papavasilieu and Miller (28) point out that, "in mammals Mn is excreted into the bile and the pancreatic juice, but not into the urine". Moav (29) reported recently that the total excretion of Mn in the urine of normal human subjects ranged from 7.7 to 16.7 μg for a 24 hour period.

TABLE V

AVERAGE NON-DIALYZABLE MANGANESE AND COPPER LEVELS IN URINE OF NORMAL AND HOSPITALIZED MALE SUBJECTS

Diagnosis	*Total* *Subjects*	*Urines*	*Creatinine* *grams/day*	*Manganese* *micrograms/day*	*Copper* *micrograms/day*
Normal	16	64	1.10 ± 0.41	0.98 ± 0.46	54.66 ± 11.67
Myocardial infarction:					
a. Questionable†	4	4	1.02 ± 0.18	1.13 ± 0.30	69.88 ± 9.98
b. Unquestionable††	4	8	1.65 ± 0.54	2.52 ± 1.15	146.87 ± 68.88
Cirrhosis of liver	1	25	1.81 ± 0.18	5.25 ± 2.55	117.26 ± 37.10
Obesity—starvation diet	1	4	2.39 ± 0.20	4.75 ± 2.59	130.78 ± 25.45
Carcinoma of the bladder	2	2	1.18	1.18	91.00
Wilsons Disease	1	1	0.98	2.92	247.50
Schizophrenia	1	1	1.07	3.29	142.84

†Urines collected 2nd day after hospital admission.

††Urines collected 2nd and 6th day after hospital admission.

The nondialyzable Mn levels in the urine of normal subjects in this study, 0.8 ± 0.3 μg per day, Table II, represent to our knowledge the first such study to be reported in the literature.

Information on the excretion of Cu and Mn in the urine of patients with myocardial infarction, cancer of the bladder, cirrhosis of the liver, schizophrenia, and starvation, is presented in Table V. Larger group of subjects in each category should be studied further in order to establish the significance of these levels. It is hoped that this initial report on Mn and Cu in the urine will serve as an impetus for further investigations of their function in health and disease. Studies on characterization of the nondialyzable Cu and Mn moieties are currently in progress in this laboratory.

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SUMMARY

Four consecutive day urine collections were made by each of 16 apparently healthy male subjects ranging in age from five to 47 years. These urines were analyzed by a neutron activation technique for nondialyzable Mn and Cu. Average concentrations obtained were 0.8 ± 0.3 μg Mn per day, and 46.0 ± 9.7 μg Cu per day.

Total Cu concentration, 50.4 ± 11.1 μg per day, was obtained by chemical analysis of these same urines.

Similarity between the mean levels for total and non-dialyzable Cu suggest that the urine excreted Cu in normal subjects is present in a bound form.

Forty-five daily urines were also obtained from 14 hospitalized adult male subjects and were analyzed for non-dialyzable levels of Mn and Cu. Manganese appears to be elevated in patients with myocardial infarction, cirrhosis of the liver, obesity (starvation diet), and schizophrenia. Copper appears to be elevated in patients with myocardial infarction and in Wilson's disease.

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