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The Treatment of Wilson's Disease with Zinc. IV. Efficacy Monitoring Using Urine and Plasma Copper (42499)

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Abstract. Progress has been made in establishing the efficacy and safety of oral zinc as a maintenance therapy for Wilson's disease. It is important to develop simple, noninvasive monitoring methods to assure the adequacy of zinc therapy in individual patients. In this paper we report the use of 24-hr urine copper and plasma copper measurements to monitor efficacy of zinc maintenance therapy in 30 Wilson's disease patients. In examples of therapeutic inadequacy such as noncompliance, these values increase. With continued long-term adequate therapy, they remain stable or decrease. These two simple monitoring tools appear to be very useful in monitoring Wilson's disease patients receiving zinc therapy.

Penicillamine is the standard treatment for Wilson's disease, an inherited disorder of copper accumulation (1–3), but has serious side effects in an appreciable proportion of patients (2, 4). For the last 6 years we have been developing zinc acetate as an alternative therapy for the maintenance treatment of Wilson's disease (4–11). Another group, in The Netherlands, has been using zinc sulfate as a treatment for Wilson's disease (12–13). Zinc acts by a different mechanism than penicillamine. The latter, through its chelating properties, causes excretion of high levels of copper in the urine. Zinc induces intestinal cell metallothionein, which then binds copper and prevents its serosal transfer into blood (14–16). The copper is lost in the stool as the intestinal cell is sloughed with a 6-day turnover.

The tools that are available to evaluate and monitor efficacy of any therapy in Wilson's disease include copper balance, liver biopsy with determination of copper concentration, plasma copper levels, 24-hr urine copper excretion, and clinical observations, including slit lamp examination for corneal copper deposits. In addition, for a therapy such as zinc which blocks intestinal absorption of copper measurement of oral ^{64}copper uptake into blood is a useful tool. All of these tools are being used to evaluate and monitor efficacy of maintenance zinc therapy in the 39 Wilson's disease patients we are treating currently. Preliminary results with copper balance (4, 8), ^{64}copper uptake (9, 10), and serial liver biopsy (11) have been reported and full papers on these topics will be published elsewhere. In general, zinc therapy has shown excellent efficacy as monitored by these methods. Also, no patient on maintenance zinc therapy has shown progression of clinical disease.

Some of the methods described above, such as copper balance, are not feasible for routine clinical monitoring. It is also a desirable goal to minimize serial liver biopsy. For monitoring efficacy of zinc therapy, it is important to develop valid, sensitive, and noninvasive procedures which are as simple as possible for general use. In this paper we report our findings concerning the use of urine and plasma copper for monitoring adequacy of zinc therapy in Wilson's disease patients.

Materials and Methods. *Patients.* This research was carried out according to the Declaration of Helsinki, informed consent was obtained in all cases, and the studies were approved by the University of Michigan Committee on the use of Humans as Research Subjects. The diagnosis of homozygous Wilson's disease was not in doubt in any of the 30 patients studied herein (Table 1). Twenty of the patients originally had presented with a typical neurological picture, 3 had presented with hepatic abnormalities only, 1 had presented with both neurological and hepatic disease, and 6 were affected but asymptomatic siblings of other Wilson's disease patients. All patients had low ceruloplasmin values and 25 had Kayser–Fleischer rings established by slit

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lamp exam at some point in their history. Twenty-six of the patients had liver biopsies, either by us or elsewhere, to help confirm the diagnosis. These biopsies were done at varying times with respect to previous penicillamine therapy, and therefore the copper levels depend somewhat on this factor. However, these data were useful in confirming the diagnosis in certain cases. For example, 4 of the 5 patients without proven Kayser–Fleischer rings had hepatic copper levels greater than 250 μg/g dry weight, a value generally agreed to be above the range of Wilson's disease heterozygotes. One patient who was not examined for Kayser–Fleischer rings at the appropriate period in his course also had never had a liver biopsy. However, he has a classical neurological picture of Wilson's, a ceruloplasmin of 1.0, and elevated urine copper even when not receiving penicillamine (207 μg/24 hr, with normal less than 50). All 4 patients who presented with hepatic manifestations had Kayser–Fleischer rings.

The patients studied comprise two groups. The larger group (group I) consists of 25 patients who previously had been treated rather extensively with copper removal therapy (penicillamine). The smaller group (group II) consists of 5 patients who have not been treated previously or had been treated inadequately and were considered to have considerable excess copper remaining. The primary zinc therapy regimen used was 50 mg of elemental zinc as the acetate salt three times per day, avoiding food by an hour before and after each dose. In patient 6 of group I and patients 20 and 36 of group II, 50 mg of zinc five times per day was used.

Urine copper and zinc. Urine for copper and zinc studies was collected for 24 hr using acid-washed trace element-free containers and urinals. Copper and zinc were assayed using an Instrumentation Laboratories Model 451 atomic absorption (AA) spectrophotometer in the flame mode. Appropriate standards were run with each set of urines.

Penicillamine cupruresis. For these studies 1 g of penicillamine in four divided doses was given for 4 days and 24-hr urines collected for the last 3 days. Zinc therapy was discontinued 3 days before and during the test period. The urines were collected and the copper assayed as described above.

Plasma copper, ceruloplasmin, and nonceruloplasmin plasma copper. For plasma copper assay, blood was collected in plastic syringes treated with zinc- and copper-free heparin, and the plasma was separated by centrifugation, diluted, and assayed by AA as mentioned above. For the ceruloplasmin assay, blood was allowed to clot and the serum was assayed for ceruloplasmin activity via the oxidation of *p*-phenylenediamine. To determine the nonceruloplasmin plasma copper, the amount of copper associated with the ceruloplasmin (3.0 μg/mg) was subtracted from the total plasma copper to derive the noncerulopasmin plasma copper (17).

Results. *Twenty-four-hour urine copper.* The urine copper data for the entire group of patients are shown in Fig. 1 and Table 2A in relation to the length of zinc therapy. The legend to Fig. 1 provides means and SD of the values in group I patients, grouped according to duration of zinc therapy. The lines in Fig. 1 connect data from the same patient. The numbers of a few patients are identified so that their data may be singled out. Patients 20, 21, 32, 36, and 41 who are identified in Fig. 1 comprise group II patients, that is, patients who had not been decoppered adequately prior to the initiation of zinc therapy. The levels of copper excretion in group II patients are higher than those of patients in group I who previously had been treated intensively with penicillamine. Further, the level of copper excretion decreased in group II patients, in some cases a dramatic decrease, as zinc treatment proceeded. Patient 21 admitted to difficulties with compliance during the midportion of his first year treatment period.

Data for urine copper excretion by particular patients in group I indicate three distinct patterns (Fig. 1). Patient 1 illustrates consistent values over the entire period of the study. A second pattern is a decline in urine copper with increasing treatment time, and therefore evidence of improved copper removal, as illustrated by patients 3 and 5. A third pattern is increasing (worsening) urine copper excretion, as shown by patient 15. Consultation with this patient revealed complicated social problems which led to a long period of institutionalization during which time the patient did not take the zinc medication. Thus, an extensive period of otherwise unreported noncompliance was

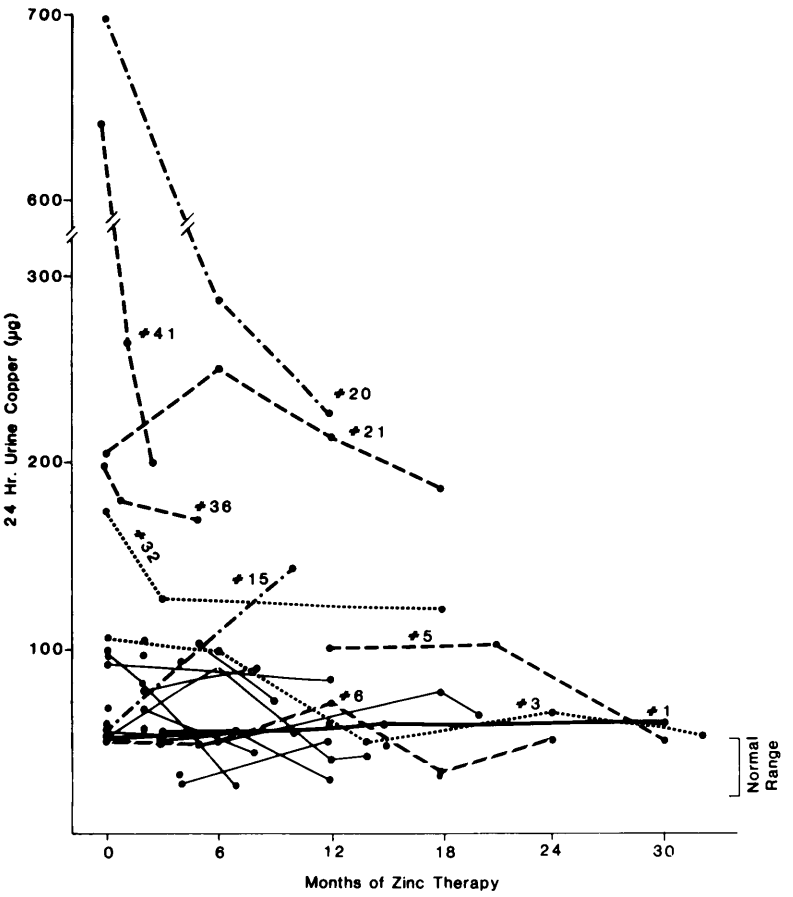

FIG. 1. Results of 24-hr urine copper assays versus months of zinc therapy in individual patients. Repeated values in the same patient are connected by lines. Values are the means of at least two sequential 24-hr collections. The means and SD of the values grouped according to duration of zinc therapy in group I patients only, given as micrograms of copper per 24 hr, are baseline, 69 ± 20, n = 10; 1–6 months, 69 ± 24, n = 15; 7–12 months, 68 ± 31, n = 13; 13–18 months, 49 ± 15, n = 7; 19–36 months, 64 ± 18, n = 7. See text for explanation of specific results singled out.

brought to light through the urine copper data of this patient. One ultimate objective of zinc maintenance therapy might be to normalize the urinary excretion of copper (the normal range if shown in brackets on the right-hand side of Fig. 1). The most recent 24-hr urine copper values in 9 of the 25 group I patients have reached the normal range (Fig. 1).

Penicillamine cupruresis. Another monitoring tool is to evaluate the amount of copper which can be mobilized for 24-hr urinary excretion in response to a standard 1 g/day dose of penicillamine. The total data are illustrated in Fig. 2 with means and SD of the group I patients, grouped according to duration of zinc therapy, reported in the legend and in Table 2B. Only one value in Fig. 2 is from a group II patient, that value being the 826 entry at the 9-month point. The data from the group I patients indicate a trend toward a decline in penicillamine cupruresis with increasing duration of zinc therapy. However, inspection of Fig. 2 in comparison with Fig. 1, and comparison of the SD in the legends of Figs. 1 and 2, reveal that there is considerably more variation in the penicillamine cupruresis data than with the 24-hr urine copper data. For example, patients 1 and 3 (Fig. 2), although showing

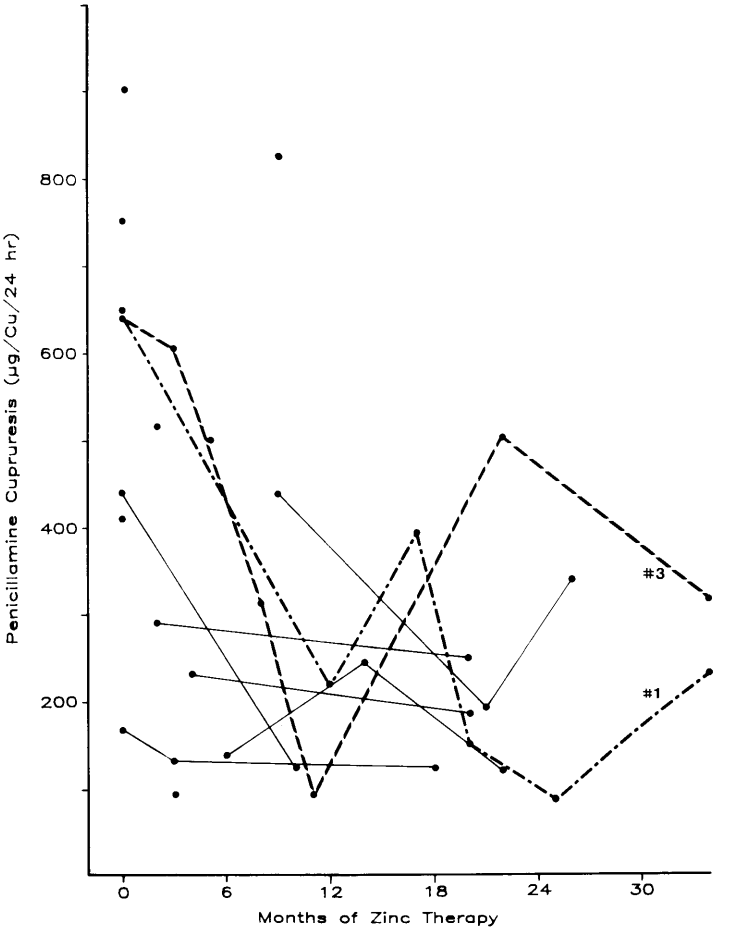

FIG. 2. Results of 24-hr urine copper assays during periods of administration of 1.0 g daily of penicillamine (penicillamine cupruresis), versus months of zinc therapy. Repeated values in the same patient are connected by lines. Values are the means of three sequential 24-hr collections. The means and standard deviations of the values grouped according to duration of zinc therapy, in group I patients only, given as micrograms of copper per 24 hours, are baseline, 536 ± 252, n = 6; 1–6 months, 315 ± 200, n = 8; 7–12 months, 449 ± 203, n = 5; 13–18 months, 255 ± 135, n = 3; 19–36 months, 243 ± 120, n = 10. See text for explanation of results singled out.

overall decreases in copper excretion with time, show considerable variation in the pattern of excretion. A graphical comparison of the means of the two variables (24-hr urine copper and penicillamine cupruresis) in relation to months of zinc therapy is shown in Fig. 3 (the SD and sample sizes associated with these means are given in the legends of Figures 1 and 2).

Zincuria. The means and SD of urine zinc excretion values of group I patients, grouped according to duration of zinc therapy, given as milligrams of zinc per 24 hr, are baseline, 0.55 ± 0.30, n = 5; 1–6 months, 5.64 ± 2.6, n = 19; 7–12 months, 6.23 ± 4.0, n = 14; 13–18 months, 4.94 ± 2.5, n = 8; 19–36 months, 5.96 ± 3.3, n = 12 (Table 2E). Thus, zincuria is dramatically increased above normal with zinc therapy but levels off at a mean of 5 to 6 mg/24 hr and does not increase progressively with increasing duration of therapy.

Plasma copper, ceruloplasmin, and noncaeruloplasmin plasma copper. Plasma copper data are illustrated in Fig. 4. Considerable variation

TABLE I. WILSON'S DISEASE PATIENTS

Patient number	Wilson's disease clinical sequelae	Ceruloplasmin value (mg/dl)	Kayser–Fleischer rings present[a]	Liver copper[b] (μg/g dry wt)	Primary zinc therapy regimen (mg/dose)	Primary zinc therapy regimen (frequency/day)
Group I						
1	Hepatic	4.2	Yes		50	3
3	Neurological	<1	Yes	183	50	2
5	Neurological	1.4	Yes	678	50	3
6	Neurological	<1	Yes	134	50	5
7	Neurological	<1	Yes	253	50	3
8	Neurological	<1	Yes	307	50	3
9	Neurological	3.3	Yes	802	50	3
10	Asymptomatic sibling	9.5	Yes	1147	50	3
12	Neurological	<1	Yes	106	50	3
14	Neurological	1.0	?		50	3
15	Neurological and hepatic	<1	Yes		50	3
16	Neurological	2.4	Yes	>0	50	3
17	Neurological	2.6	Yes	718	50	3
18	Neurological	<1	Yes	112	50	3
19	Neurological	<1	Yes	1224	50	3
22	Neurological	4.8	Yes	87	50	3
24	Neurological	<1	Yes	510	50	3
25	Asymptomatic Sibling	1	No	491	50	3
26	Neurological	2.9	Yes		50	3
27	Asymptomatic Sibling	<1	No	502	50	3
28	Hepatic	<1	Yes	2468	50	3
30	Neurological	14.9	Yes	345	50	3
31	Asymptomatic Sibling	<1	No	700	50	3
33	Hepatic	<1	Yes	310	50	3
34	Neurological	<1	Yes	261	50	3
Group II						
20	Neurological	<1	Yes	428	50	5
21	Asymptomatic sibling	3.8	No	493	50	3
32	Asymptomatic sibling	10.8	Yes	639	50	3
36	Neurological	<1	Yes	870	50	5
41	Neurological	<1	Yes	505	50	3

[a] This entry refers to Kayser–Fleischer rings being documented by slit lamp exam at some time in patient's history, not necessarily at time of this study.

[b] This entry indicates the highest hepatic copper value known to us, either from our own biopsy, or from records of another institution. Since many of these biopsies were done after considerable penicillamine therapy in Group I patients, they reflect the fact that considerable copper had already been removed.

occurs in individual patients (note in particular patients 5 and 6). The means and SD of plasma copper in group I patients, grouped according to duration of zinc therapy, are given in the legend. There is an overall trend toward decreasing plasma copper as treatment proceeds, but this has not reached statistical significance.

A small part of the decrease in plasma copper is due to a gradual decrease in ceruloplasmin as shown in individual patients in Fig. 5, with the means and SD listed in the legend. Again this decrease is not statistically significant at this point. When ceruloplasmin copper is substracted from the plasma copper, as dis-

TABLE II. MONTHS OF ZINC THERAPY (GROUP I PATIENTS)

	0	1–6	7–12	13–18	19–36
A. 24-hr urine copper excretion (means ± 1 SD)					
(μg/24 hr)	69 ± 20	69 ± 24	68 ± 31	49 ± 15	64 ± 18
N	10	15	13	7	7
B. Penicillamine cupruresis (means ± 1 SD)					
(μg/24 hr)	536 ± 252	315 ± 200	449 ± 463	255 ± 135	243 ± 120
N	6	8	5	3	10
C. Zincuria (means ± 1 SD)					
(mg/24 hr)	0.55 ± 0.30	5.64 ± 2.6	6.23 ± 4.0	4.94 ± 2.5	5.96 ± 3.3
N	5	19	14	8	12
D. Plasma copper (means ± 1 SD)					
(μg/dl)	25.0 ± 21	23.7 ± 15	28.6 ± 18	22.1 ± 8	18.0 ± 8
N	12	16	13	7	8
E. Ceruloplasmin (means ± 1 SD)					
(mg/dl)	4.9 ± 3.6	3.6 ± 3.8	2.6 ± 2.0	2.4 ± 1.3	2.7 ± 1.5
N	13	14	12	6	8
F. Nonceruloplasmin plasma copper (means ± 1 SD)					
(μg/dl)	12.3 ± 12.8	16.3 ± 14.0	23.0 ± 14.0	13.2 ± 6.0	10.1 ± 9.6
N	12	14	12	6	8

cussed in Materials and Methods, the result is nonceruloplasmin plasma copper (Fig. 6 means and standard deviations in Table 2). There is considerable variation in this variable, even with individual patients, over short periods of time. (See in particular patients 5 and 6 of Fig. 6.) However, there does appear to be a downward trend in the overall plasma copper values after the first year of therapy, as suggested by the decreasing means over time given in the legend. This trend is not statistically significant at this time. Patients 5 and 6 had quite high nonceruloplasmin plasma coppers at the 12-month point. Patient 5 admitted a compliance problem during that period which was later resolved. Patient 6, on the other hand, claims to have complied well with her therapy; however, additional evidence not reported here (oral ^{64}copper uptake into blood) supports the view that her copper uptake was not under adequate control. She had been on a zinc regimen of 25 mg four times a day plus 50 mg at bedtime for a total daily dose of 150 mg. Her dosage of zinc was increased to 50 mg 5 times per day (250-mg daily dose). Her nonceruloplasmin plasma copper came down (Fig. 6) and her 24-hr urine copper decreased (Fig. 1). That control of her copper uptake was now adequate was supported by an oral ^{64}copper uptake study at this point and a copper balance which was not negative (data not reported here). This case illustrates not only the usefulness of following nonceruloplasmin plasma copper over time, but reemphasizes the usefulness of following 24-hr urine copper excretion.

Discussion. Zinc therapy is being developed as an alternative maintenance therapy for Wilson's disease (4–13). Standard chelation therapy with penicillamine acts by increasing copper excretion in the urine (1–3), and efficacy is usually monitored by following 24-hr urinary copper. Zinc acts through a different mechanism, increasing the fecal excretion of copper (14–16). Because of its different mode of action, new methods must be developed and validated for monitoring the efficacy of zinc therapy. Some of the possible monitoring tools, such as copper balance, liver biopsy with copper measurements, and oral ^{64}copper uptake into blood, while excellent are difficult to use in a nonresearch setting. The main thrust of the work reported in this paper was to evaluate the usefulness of various urine and blood copper measurements to monitor the efficacy of zinc maintenance therapy in Wilson's disease.

The first point we would like to stress is that 24-hr urine copper excretion appears to be an extremely useful procedure. In the usual sit-

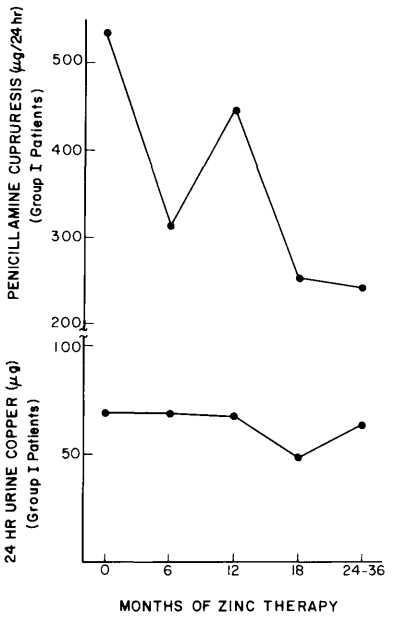

FIG. 3. This figure presents the means and SD of 24-hr urine copper and penicillamine cupruresis data presented in Figs. 1 and 2, respectively. The data for 6-month intervals are pooled, and the means are presented. For the sample size used to determine each point, please consult the legends to Figs. 1 and 2.

uation, in penicillamine-treated Wilson's disease, the amount of copper excreted in the urine relates to two factors. These are, first, the amount of mobilizable copper load in the body available for excretion, and, second, the effectiveness and dose of the penicillamine therapy. Since two factors are involved, one cannot use the urinary copper excretion solely as a measure of excessive copper load, although it is usually somewhat indicative. However, in the absence of penicillamine therapy (as is the case with zinc therapy), the quantity of copper in the urine becomes, as with glycosuria in a diabetic, a reflection of the level of excess copper. Thus, we see with treatment, as in Fig. 1, a fairly rapid decline of urine copper in previously untreated patients, and a slower decline toward the normal range in patients who previously had been treated with copper removal therapy. Further, we see in patient 15, and to a lesser extent in patient 6, the usefulness of the change in excretion over time in detecting potential problems.

Penicillamine cupruresis in this setting (zinc-treated patients) seems to us to be relatively less useful. First there is the disadvantage

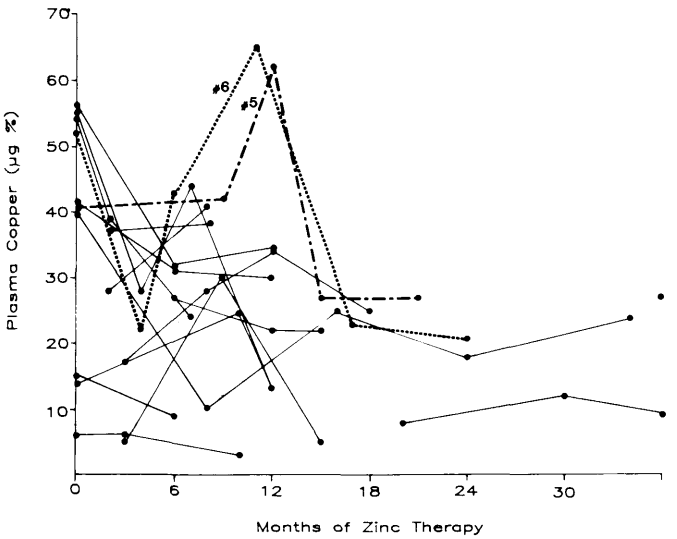

FIG. 4. Results of plasma copper assays versus months of zinc therapy. Repeated values in the same patient are connected by lines. The means and SD of the values grouped according to duration of zinc therapy, given as microgram percentages are baseline, 25.0 ± 2.1, n = 12; 1–6 months, 23.7 ± 15, n = 16; 7–12 months, 28.6 ± 18, n = 13; 13–18 months, 22.1 ± 8, n = 7; 19–36 months, 18.0 ± 8, n = 8. See text for explanation of results singled out.

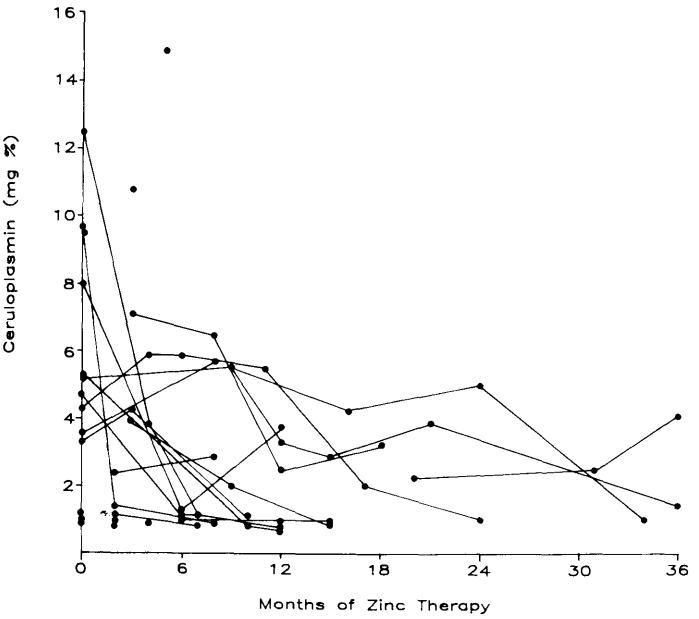

FIG. 5. Results of ceruloplasmin assays versus months of zinc therapy. Repeated values in the same patient are connected by lines. The means and SD of the values, grouped according to duration of zinc therapy, given as microgram percentages are baseline, 4.9 ± 3.6, n = 13; 1–6 months, 3.6 ± 3.8, n = 14; 7–12 months, 2.6 ± 2.0, n = 12; 13–18 months, 2.4 ± 1.3, n = 6; 19–36 months, 2.7 ± 1.5, n = 8.

that penicillamine must be reinstituted temporarily with the risk of hypersensitivity reactions. Second, as illustrated in Fig. 2, the cupruresis data are quite "noisy" compared with the 24-hr urine copper data. Third, there is a theoretical problem in interpreting the data during increasing length of zinc therapy. The decline in penicillamine cupruresis illustrated in Fig. 3 could be explained by at least two possible mechanisms. The first would be increasing control and lowering of copper burden by therapy, and second, an increasing body load of zinc resulting in more of the penicillamine being involved in provoking zincuresis and less available for cupruresis. That this latter factor may be important is suggested by comparison of the two lines in Fig. 3 summarizing the means of 24-hr urine copper and cupruresis. Twenty-four-hour urine copper decreases only very gradually with progression of zinc therapy in group I patients, while cupruresis decreases more dramatically. The greater decrease in cupruresis may reflect the increasing effect of zinc on interfering with penicillamine's effectiveness in causing cupruresis. Because of these real and theoretical disadvantages, we are no longer using penicillamine cupruresis in zinc-treated patients as an evaluative tool.

The zincuria data illustrate that the excretion of zinc in the urine, while dramatically increased over normal, stabilizes at an average value of about 5 to 6 mg per 24 hr. These data are reassuring in the sense that there is not a progressive increase in zincuria as zinc therapy proceeds. The zincuria in a zinc-treated patient is also helpful in evaluating compliance. If the 24-hr urine zinc value is below 3.0 mg in a patient treated with 150 mg/day of zinc after several months of therapy, a problem with compliance should be considered.

The subtraction of the ceruloplasmin copper from the total plasma copper yields the variable, nonceruloplasmin plasma copper. This more loosely bound copper is presumably the copper that causes toxicity in Wilson's disease, and therefore controlling its level is important. The range of non-ceruloplasmin plasma copper of normal subjects is approximately 10–20 μg%. Thus, one objective of

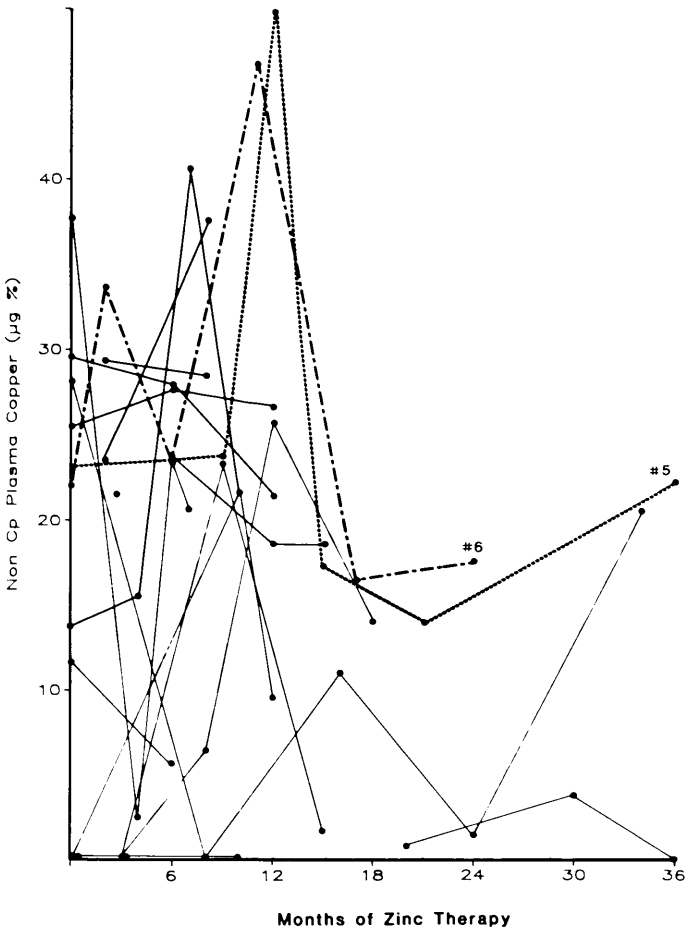

FIG. 6. Results of nonceruloplasmin plasma copper determinations derived by subtracting ceruloplasmin copper from plasma copper (see Materials and Methods) versus months of zinc therapy. Repeated values in the same patient are connected by lines. The means and SD of the values, grouped according to duration of zinc therapy, given as microgram percentages are baseline, 12.3 ± 12.8, n = 10; -16 months, 16.3 ± 14.0, n = 14; 7-12 months, 23.0 ± 14, n = 12; 13-18 months, 13.2 ± 6, n = 6; 19-36 months, 10.1 ± 9.6, n = 8. See text for explanation of results singled out.

therapy is to reduce the nonceruloplasmin plasma copper to at least 25 µg%. In individual patients, as treatment with zinc progresses, this variable tends to decrease to that value or below. During temporary problems with compliance or other reasons for lack of copper control, this variable increases, and is therefore useful in the continued monitoring of the patient.

Thus, of the variables investigated here, we believe that 24-hr urine copper and nonceruloplasmin plasma copper are the most useful in monitoring zinc control of copper in Wilson's disease. At the present time, all of our patients are also being monitored by copper balance and ^{64}copper uptake, and as many as possible by serial liver biopsy with copper assay. However, ultimately, copper balance and liver biopsy copper will not be practical monitoring tools if zinc is to be used in other than a research setting. Based upon our work so far, it appears that the physician may be able to use 24-hr urine copper excretion, nonceruloplasmin plasma copper, and ^{64}copper uptake as adequate tools for both the initiation and monitoring of zinc therapy in this disease.

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