nm/PRELIMINARY NOTE

# DETECTION OF THE HETEROZYGOTE OF WILSON'S DISEASE

S. O'Reilly, P. Weber, M. Pollycove and L. Shipley

*San Francisco General Hospital, University of California School of Medicine, San Francisco, California*

Although one may accept Penrose's axiom that "it is not respectable to discuss a homozygous trait without mentioning the findings in the carrier" (*1*), so far identification of the heterozygote in Wilson's disease has not been uniformly successful.

Previous reports have indicated that one or more abnormalities in relation to the physiology of copper may be found in presumptive heterozygotes: for example, subnormal levels of ceruloplasmin (*2*), increase in nonceruloplasmin copper (*3*) and decreased incorporation rate of radiocopper into ceruloplasmin (*4*) and into erythrocuprein, the red cell copper protein (*5*).

Nevertheless, with most of these measurements there was a variable degree of overlap between normal subjects and presumptive heterozygotes (*6*). We wish to present evidence that it may be possible to separate more clearly the heterozygote from the normal subject and identify the carrier by measuring the turnover of copper in the whole body using a whole-body counter and a longer-lived isotope of copper than has been available before.

The studies were carried out in six normal control subjects, three known homozygotes and two presumptive heterozygotes of Wilson's disease, and in two siblings of one of the known homozygotes. One of these siblings was judged to be normal and the other heterozygous on the basis of their whole-body turnover and other studies. Two separate studies were made on one of the homozygotes.

Each subject in the fasting and resting state was given from 15 to 127 $\mu$Ci of virtually carrier-free $^{67}$Cu (physical half-life approximately 61 hr) which had been previously incubated with 10 ml of the subject's plasma to insure protein-binding of the tracer. In each case background radioactivity in the whole body had been determined before injection.

If no excretion took place, the absorbed whole-body dose would be less than 0.49 mrads/$\mu$Ci injected, or between 7 and 62 mrads in the dose range used. There is therefore no radiation hazard in such studies.

Each subject was counted daily or every other day in the whole-body counter for up to 3 weeks. Excreta were collected and counted from most of the subjects, and the results confirmed the validity of using the whole-body-counter data to determine total-body turnover and retention. Details of instrumentation and methodology will be given in a more complete communication at a later date. The half-times for turnover of copper in the whole body are seen in the table.

Since the patients could only be followed for about 21 days, it is not possible to be certain that there is no additional pool with a slower turnover, particularly in the Wilsonian homozygotes. Thus, strictly speaking, these reported half-times may not be biologic half-times.

Figure 1 shows whole-body measurements in one normal, one homozygous and one heterozygous subject to illustrate the kind of data from which the half-times were derived. Subject MG was thought to be a heterozygote on the basis of the prolonged turnover, and this tended to be confirmed by finding reduced incorporation of radiocopper into ceruloplasmin and erythrocuprein in the detailed studies of copper kinetics carried out during the biological turnover of the radiocopper. His sister (TG), on the other hand, was judged to be homozygously normal since her half-time fell well within the normal range and she incorporated radiocopper normally into specific copper proteins.

It is evident from the table that the mean half-time for seven normal subjects was 26 days with a range of 22–33 days. The three homozygotes had half-times of 129, 104–108 and 185 days while the presumptive heterozygotes had half-times of 73, 44 and 60 days. A clearcut separation between normal, homozygote and heterozygote is suggested by these half-times.

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For reprints contact: Sean O'Reilly, San Francisco General Hospital, 22nd and Poterero St., San Francisco, Calif. 94110.

**TABLE 1. HALF-TIMES FOR COPPER TURNOVER**

| Subjects | Age (yr) | Sex | Biological half-time $^{67}Cu$ (days) |
|---|---|---|---|
| | *Normal* | | |
| TO'R | 13 | M | 23 |
| WF | 13 | M | 30 |
| SO'R | 45 | M | 25 |
| LF | 16 | F | 22 |
| SF | 15 | F | 33 |
| JQ | 29 | F | 30 |
| TG | 21 | F | 22 |
| | *Known homozygotes* | | |
| BG | 13 | M | 129 |
| SG | 20 | F | 108 |
| (2 studies) | | | 104 |
| CO | 16 | F | 185 |
| | *Presumptive heterozygotes* | | |
| RG | 46 | M | 73 |
| VG | 43 | F | 44 |
| MG | 16 | M | 60 |

If prolonged turnover of copper is a consistent and distinctive finding in the heterozygote of Wilson's disease, then the abnormal allele must be related to whatever factor is responsible for the delayed turnover. The next step must then be to identify, if possible, the responsible factor, in the hope of "catching the product of the abnormal allele as it comes off the ribosome," to use Bearn's apt phrase (7).

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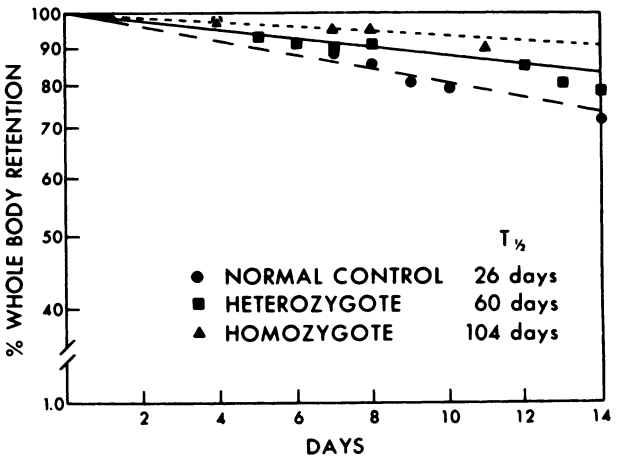


**FIG. 1.** Whole-body retention of $^{67}Cu$ in Wilson disease heterozygotes and homozygotes and in normal controls.

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