Biochemical validation of a self-administered semi-quantitative food-frequency questionnaire

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The present study is a biochemical validation of a food-frequency questionnaire (FFQ) with optical reading, i.e. containing food portion photographs to help to assess quantities. Forty-four healthy subjects, non-smokers and not taking vitamin supplements, were recruited for the study. After completion of the questionnaire, subjects were asked to keep a 7 d weighed dietary record (7DR). Three 24 h urine samples were collected on 3 different days over the week of food recording for the analysis of urea-N, P and K. On the 4th day of food recording, blood was collected for determination of \( \alpha \)-tocopherol, \( \beta \)-carotene and ascorbic acid. N, P and K determined in urines and from 7DR were significantly correlated (Spearman rank correlation test), \( r \) values being 0.77, 0.57 and 0.42 respectively. The correlations with the FFQ were significant only for N (\( r \) 0.45) and P (\( r \) 0.39). Blood ascorbic acid and \( \beta \)-carotene concentrations correlated with dietary intake when determined from 7DR (both \( r \) 0.44), but not when determined from FFQ. No correlation was found for \( \alpha \)-tocopherol. The data obtained seem to prove the validity of the FFQ in defining eating patterns in terms of some nutrients, but not vitamins, at least as far as non-supplemented subjects are concerned. The way in which foods were grouped in the questionnaire could account for these results.

Biochemical validation: Food-frequency questionnaire: Food intake.

The main purpose of nutritional epidemiology is to study the relationship between an individual’s habitual food intake and the risk of disease (Willett, 1990). The food-frequency questionnaire (FFQ) method, notwithstanding some limitations, has become the preferred method in nutritional epidemiology (Willett, 1990), being of low cost and easy to administer. Although the various FFQ have progressively improved their capability of measuring dietary intake, some consistent limits still exist especially due to the lack of quantitative information. In line with this we have developed a self-administered semi-quantitative FFQ which contains food portion photographs (Fidanza et al. 1994) to help to assess quantities. Its biochemical validation is reported here.

Considering that all the methods used for dietary appraisal of free-living subjects are in general affected by systematic errors (Bingham, 1987), it is impossible to have a valid and reliable reference method for nutrient intake. So to assess the validity the results of one method are compared with another which has a greater acceptance or can be considered more accurate, and until now the weighed record has generally been considered the ‘gold standard’ (Willett et al. 1985; Block et al. 1990; Hankin et al. 1991). The major limitation
of this form of validation is that the measurement errors of the two methods are not completely independent.

Biochemical markers may offer independent validation of nutrient intake, on the condition that they are sensitive to dietary intake. Suitable markers are not available for many nutrients, but Isaksson (1980) suggested that 24 h urine-N excretion could provide an objective measure of the habitual protein intake of free-living individuals, and since then different studies have demonstrated that it can be used as a validatory method because it is independent of techniques used to assess dietary intake (Bingham & Cummings, 1985; Maroni et al. 1985; Gentile et al. 1987; D'Amico & Gentile, 1990). Not much information is available on the use of urinary P, but we know that in normal adults intestinal P absorption and urinary excretion of phosphate are about 75% of the dietary intake, whereas with a dietary intake lower than 2 mg/kg per d, 80–90% of ingested P is absorbed and excreted in the urine (Wilkinson, 1976). In contrast, urinary K has been used frequently as a marker of dietary intake (Blackburn & Thornton, 1979; Caggiula et al. 1985; Castenmiller et al. 1985; Fregly, 1993).

Blood values for several vitamins are considered to reflect intake (Willett et al. 1983b) despite the inevitable confusion created by individual variability in absorption, availability and metabolism. Serum β-carotene and α-tocopherol have been used for evaluating the validity of dietary questionnaires in previous studies (Willett et al. 1983a; Romieu et al. 1990; Bolton-Smith et al. 1991; Jacques et al. 1993). The measurement of fasting plasma ascorbic acid was also reported (Bates, 1991) to be a reasonably reliable index of ascorbic acid status for population studies and was proposed as a biochemical marker for studying the validity of questionnaires (Romieu et al. 1990; Jacques et al. 1993). Unfortunately, until now, validation studies with blood levels of vitamins have principally been done in groups of people that include supplement users, making it difficult to understand their validity in non-supplemented populations.

For these reasons we validated our FFQ with both weighed records for 7 d and biochemical variables in urine (urea-N, P and K) and in blood (β-carotene, α-tocopherol and ascorbic acid), even though for the last variables no definitive data are present in the literature.

METHODS

Subjects

The participants were recruited from the students and technicians attending the Agriculture Faculty and the Nursing School of San Carlo Hospital of Milan. Thirty-eight subjects out of the forty-four recruited completed all the required parts of the study, while six omitted the blood collection. All the subjects (eleven males and thirty-three females, mean age 27.1 (SD 6.3) years) were in the range of reference weight-for-height (BMI 21.1 (SD 2.7) kg/m²), non-smokers, not suffering from any chronic disorder or following any special diet. None used vitamin supplements.

Procedure

The experimental period lasted 8 d. The study design is summarized in Fig. 1. On the first day the participants were invited to the laboratory and their weight, height, systolic and diastolic blood pressure values were registered. Then they received the FFQ and instructions for its completion. The semi-quantitative FFQ (Fidanza et al. 1994) consists of a list of ninety-three foods among the most commonly consumed in Italy. The different items are grouped into sixty-one classes according to their similarity in relevant nutrients and their customary use. For each food class colour photographs of three different portions are displayed beside the relevant questions. Portion sizes were chosen according to many years of experience in dietary surveys in various parts of Italy. Food frequency is evaluated
Using three categories: daily, weekly and monthly and from 1 to 6 number of times (for example: once a day, 3 times a week, 6 times a month). The questionnaire has to be completed by filling in the appropriate boxes with a pen, so no writing is required.

On the second day the subjects returned the questionnaire and were given appropriate recording forms and instructions to keep a 7 d weighed dietary record (7DR). They were also provided with electronic scales (Soehnle, Germany) to quantify the food portion consumed and were instructed to use household measures when away from home (Fidanza et al. 1994). Subjects were also asked to provide detailed descriptions of each food, including brand and method of preparation and recipes whenever possible.

Food and nutrient intakes from 7DR and FFQ were computed by the dietitian using the same database system. The nutrient database was compiled from food composition tables (Fidanza & Versiglioni, 1989) plus partly published data on cooked foods (Porrini et al. 1986).

The 24 h urine samples were collected at the beginning of the record period, after 3 d, and on the last day, in 2.5-litre plastic jugs. The 24 h collection started with the first morning specimen and lasted until the night. Each 24 h collection was returned to the laboratory on the following day and immediately analysed. All the subjects were instructed about the necessity of a complete urine collection; an apposite written form was prepared. To check the completeness of 24 h urine collections the 24 h creatinine excretion of each subject was compared with published values specific for height and sex (Blackburn & Thornton, 1979).

Blood was taken when the subjects returned the second 24 h urine collection; they had been asked to fast from 22.00 hours the previous evening. Blood (4 ml) was collected in a 10 ml tube containing 0·1 ml EGTA-glutathione as anticoagulant and protected from light. A 1 ml portion was transferred into 4 ml 0·3 M-TCA, vortexed and centrifuged for 10 min at 3000 g; the supernatant fraction was used for ascorbic acid determination. The remaining 3 ml of whole blood was centrifuged for 10 min at 800 g to separate plasma from erythrocytes. Plasma was used for β-carotene and α-tocopherol determination.

**Potassium, phosphorus, urea-nitrogen and creatinine determination in urine**

K in urine was determined by a potentiometric method (Dahl et al. 1972). P and urea-N were determined by enzymic methods (Griswold et al. 1951; Whelton et al. 1994). From urea-N it was possible to estimate dietary protein intake, considering that in a steady-state condition (i.e. zero net anabolic/catabolic rate) the total N generation by protein
catabolism is closely related to protein (N) intake and to urinary non-urea-N and faecal N (NUN), which is dependent on body weight. The dietary protein intake was estimated from urea-N using the equation developed by Maroni et al. (1985):

\[
\text{protein intake (g/d)} = 6.25 \times (\text{UUN} + 0.031 \times \text{BW})
\]

where UUN is urine urea-N (g/d) and BW is body weight (kg).

This relationship has been previously validated (Gentile et al. 1987; D'Amico & Gentile, 1990) allowing excretion of extrarenal N (faecal and dermal losses) to be corrected to the body weight of the subjects.

Creatinine was determined by an enzymic test (Bonsens & Taussky, 1945).

\section*{β-Carotene and α-tocopherol determination in plasma}

Extraction of β-carotene and α-tocopherol from plasma was carried out as described by Vuilleumier et al. (1983). The analysis was performed by HPLC using a Waters 510 pump (Hillford, MA), Varian 9050 spectrophotometer detector (Walnut Creek, CA), Spectra Physics Work Station (San Jose, CA) and Olivetti P500 computer (Ivrea, Italy).

The separation was performed on a 4 mm i.d. \times 250 mm column packed with 7 \(\mu\)m Lichrosorb Si60 (Merck, Darmstadt, Germany) protected by a 4 mm i.d. \times 4 mm guard column (Merck).

The operating conditions for β-carotene analysis were: n-hexane–dioxane (1000:5, v/v), isocratic, 1.2 ml/min, spectrophotometric detection 453 nm. The CV of duplicate assay was 2.6%.

The operating conditions for α-tocopherol analysis were: n-hexane–ethyl acetate (1000:75, v/v), isocratic, 2.0 ml/min, spectrophotometric detection 294 nm. The CV of duplicate assay was 2.2%.

As α-tocopherol plasma levels are highly correlated with total lipids and cholesterol (Vuilleumier et al. 1991), the ratios of plasma tocopherol to triacylglycerols and cholesterol were also considered.

\section*{Ascorbic acid determination in blood}

After deproteinization of whole blood and enzymic oxidation of L-ascorbic acid to dehydro-L-ascorbic acid the latter was condensed with o-phenylenediamine to its quinoxaline derivative. The derivative was separated by HPLC (Speek et al. 1984) using an isocratic Waters 510 pump, Perkin Elmer LS-1 fluorimeter and 4 mm i.d. \times 250 mm column packed with 5 \(\mu\)m Aluspher RP-select B (Merck). The operating conditions were: 0.08 M-KH\(_2\)PO\(_4\)–methanol (80:20, v/v), pH 7.8, isocratic, 1.0 ml/min, spectrofluorimetric detection, 304 nm emission and 425 nm excitation. The CV of duplicate assay was 3.6%.

\section*{Statistical analysis}

For descriptive purposes, means and standard deviations of dietary nutrient intakes and blood and urine variables were calculated. As the variables we studied were not all normally distributed using the Shapiro–Wilks' W test, the associations of nutrient intakes with blood and urine variables were examined using Spearman's rank correlation test. The nutrient values determined from the dietary methods and from urine were compared using Wilcoxon's matched-pairs signed rank test. The ability of the FFQ and the 7DR to assign individuals correctly into tertiles of blood vitamin concentration was also assessed. Statistical tests were performed using Statistica by StatSoft Inc. (Tulsa, OK).

\section*{RESULTS}

The comparison of nutrient intake determined from the FFQ and the 7DR has been reported in a previous paper (Fidanza et al. 1994).
Table 1. Urine variables determined from 24 h collections, and daily nutrient intakes determined from a food-frequency questionnaire (FFQ) and a 7 d weighed record (7DR) for forty-four subjects

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Urine</th>
<th>FFQ</th>
<th>7DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/d)</td>
<td>68</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>Phosphorus (mmol/d)</td>
<td>28.4</td>
<td>41.6</td>
<td>35.3</td>
</tr>
<tr>
<td>Potassium (mmol/d)</td>
<td>53.8</td>
<td>63.7</td>
<td>61.1</td>
</tr>
</tbody>
</table>

* Wilcoxon’s rank test to assess the difference between the methods: protein in urine v. FFQ P = 0.567, protein in urine v. 7DR P = 0.866.

Table 2. Blood levels and daily intake of selected vitamins determined from a food-frequency questionnaire (FFQ) and a 7 d weighed record (7DR) in thirty-eight subjects

(Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Variable</th>
<th>FFQ</th>
<th>7DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg/l)</td>
<td>9.9</td>
<td>2.6</td>
</tr>
<tr>
<td>β-Carotene (μg/l)</td>
<td>391</td>
<td>192</td>
</tr>
<tr>
<td>α-Tocopherol (μg/ml)</td>
<td>11.5</td>
<td>2.5</td>
</tr>
<tr>
<td>FFQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg/d)</td>
<td>108.7</td>
<td>49.2</td>
</tr>
<tr>
<td>β-Carotene (μg/d)</td>
<td>3569.5</td>
<td>1308.8</td>
</tr>
<tr>
<td>α-Tocopherol (mg/d)</td>
<td>4.4</td>
<td>1.0</td>
</tr>
<tr>
<td>7DR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (mg/d)</td>
<td>86.4</td>
<td>39.7</td>
</tr>
<tr>
<td>β-Carotene (μg/d)</td>
<td>2653.2</td>
<td>1245.2</td>
</tr>
<tr>
<td>α-Tocopherol (mg/d)</td>
<td>3.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* For details of procedures, see pp. 324–326.

In Tables 1 and 2, mean values and SD of urine and plasma variables and the mean nutrient intake and SD determined from the FFQ and the 7DR are reported. Urine collections in which the total amount of creatinine was ± 15% of the expected amount were considered incomplete and rejected, and this left 100 collections out of 130 (77%). This percentage is acceptable considering the results obtained in other similar studies: Petersen et al. (1992), after considering only one urine collection and checking completeness of the collection using para-amino-benzoic acid (PABA) (Bingham & Cummings, 1983), rejected 35% of the samples; O’Donnell et al. (1991) should have rejected 65% of collections, yet they decided to include all data in the statistical analysis.

Protein intakes determined from the FFQ and the 7DR and estimated from urine were not statistically different using the Wilcoxon matched-pairs signed rank test.

Under normal conditions the amount of P absorbed depends, among other things, on its concentration in the diet (Wilkinson, 1976; Krane & Potts, 1983). Assuming that the amount excreted is about 75% of P ingested, the mean value obtained from the urine of our subjects was comparable with the level of intake determined with both the dietary methods.
Table 3. Spearman rank correlation coefficients (r) between nutritional variables determined from 24 h urine collections and the same variables determined from a food-frequency questionnaire (FFQ) or a 7 d weighed dietary record (7DR) in forty-four subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Urine v. FFQ</th>
<th>Urine v. 7DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>0.453</td>
<td>0.769</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.389</td>
<td>0.566</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.176</td>
<td>0.417</td>
</tr>
</tbody>
</table>

Table 4. Spearman rank correlation coefficients (r) between blood levels of vitamins and their dietary intake determined from a food-frequency questionnaire (FFQ) or a 7 d weighed dietary record (7DR) in thirty-eight subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blood v. FFQ</th>
<th>Blood v. 7DR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>+0.069</td>
<td>+0.437</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>-0.068</td>
<td>+0.439</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>-0.220</td>
<td>+0.498</td>
</tr>
</tbody>
</table>

Considering faecal K excretion to be from 5 to 10 mmol/d (Man, 1994), the urinary K excretion nearly equalled K intake determined from the 7DR, while the intake from the FFQ was higher.

Spearman’s correlation coefficients and significance between urine variables and dietary intake assessed from FFQ and 7DR are reported in Table 3, while correlation coefficients and significance between plasma variables and dietary intake are reported in Table 4. Urine and blood variables were well correlated with nutrient intakes from 7DR, with the exception of α-tocopherol. When nutrient intake was determined from the FFQ only protein and P were significantly correlated. Plasma α-tocopherol levels did not correlate with dietary intake even when they were considered in relation to cholesterol and triacylglycerol levels.

The ability of the dietary methods to assign people correctly into tertiles of blood vitamin concentrations is reported in Table 5. The percentage of correct classification was higher for 7DR than for FFQ, the lowest values being for α-tocopherol. Altogether the percentage of misclassification varied from 5 to 32%.

**DISCUSSION**

There have been numerous studies to investigate the validity and reliability of FFQ (Willett et al. 1983a, 1985, 1987; Block et al. 1990; Munger et al. 1992; Rimm et al. 1992) and it can be concluded that they are good instruments for nutrient-intake assessment. In our opinion, because of the limited number of food items reported and because of the semi-quantitative nature of FFQ, quantitative interpretation of values derived from the questionnaires is difficult, especially when micronutrients are considered. The validation
study described here together with the data previously reported (Fidanza et al. 1994) show that the semi-quantitative FFQ can be considered to be a useful method for evaluating food intake and ranking subjects according to intakes of certain nutrients.

Concerning the validation protocol we employed, as discussed in the previous paper (Fidanza et al. 1994), a major problem in the use of the weighed record as a reference method is the fact that 1 week cannot fully represent the usual intake. On the other hand, from our experience, prolonging the record period can make the accuracy of the records worse and induce subjects to make subconscious changes to their dietary habits.

To improve the accuracy of the validation study and to have an independent validatory measure of dietary intake, we also considered some biological markers of food intake in urine and blood. A problem with the use of urine is to ensure the completeness of collections. A method commonly used to validate the completeness of 24 h collections in humans is the determination of creatinine excretion, even though it varies among individuals (Jackson, 1966) and from day to day when the diet is not constant (Vestergaard et al. 1958). This variability is caused by differences in the creatinine pool size and by the influence of creatinine in meat on the creatinine pool. Notwithstanding this, creatinine excretion has already been used by numerous authors in different studies (Caggiula et al. 1985; Castenmiller et al. 1985; D'Amico et al. 1992; Gentile et al. 1993).

To overcome the problem of variability we also planned three 24 h urine collections on 3 different days during the week of food recording and rejected the urine collections that were incomplete. Good agreement was found between the daily protein intake estimated from the FFQ and the 7DR and the daily intake estimated from the 24 h urine excretion.

Besides protein determination we thought it would be useful to evaluate P and K, considering that the principal route for excretion of these two elements is in the urine and that, due to their wide distribution in many different kind of foods, they could be useful markers of food intake.

O'Donnell et al. (1991) in a validation study of a FFQ found results similar to ours for K and N from 24 h urine and from 16 d weighed records. The correlations they found between urine and FFQ were significant only for protein. The authors concluded that because of 'the poor-to-moderate associations between the questionnaire and biochemical variables, the questionnaire might not be powerful enough to detect possible relationships between diet and disease mediated by blood biochemistry'. However, they confirmed the validity of urinary N as a biochemical measure for dietary protein. From our results it
seems possible to say that urine K and P are also useful indices of intake, and should be considered with more attention in protocols of validation.

We did not find statistically significant correlations between vitamin intake determined from the FFQ and plasma levels. The classification into tertiles revealed a moderate agreement between the circulating levels of vitamins and their intake in the diet determined from the questionnaire. In fact a 33% correct tertile classification and 22% gross misclassification would be expected as a result of chance alone. For α-tocopherol it has been reported (Vuilleumier et al. 1983) that even if there is no homeostatic mechanism regulating its concentration in blood, the correlation between intake and plasma level should be expected principally in the low intake range. Consequently, in a well nourished population a lack of correlation could be easily explained. On the contrary, in a previous study (Porrini et al. 1987) we found a correlation between ascorbic acid intake and its plasma levels (r = 0.50) in an unsupplemented elderly population.

In contrast to our data, Jacques et al. (1993) demonstrated that the FFQ they validated provided useful and valid information on micronutrient intake for pteroylglutamate (folate), vitamin C, vitamin D, vitamin B₁₂ and vitamin E for both sexes, and β-carotene for females. Two principal points differentiate their study from ours: they included supplement users, and this fact strengthened most associations between the FFQ and biochemical variables. This happened clearly because supplement users are more accurately classified according to intake and, in addition, both the range of intake and of the biochemical measure is expanded, improving the correlation between reported intake and biochemical measure. In the population that they studied, 43% of the subjects used some form of nutrient supplement, a percentage comparable with that in the US population. Italian people are not used to taking vitamin supplements, and in our subjects no vitamin supplement users were present. Furthermore, even though the questionnaire was designed to be self-administered, in Jacques' validation study it was administered by interview. Other investigators (Leighton et al. 1988) have pointed out substantial differences in data quality between self-administered and interviewer-administered questionnaires.

Coates et al. (1991) studied the validity of the Health Habits and History Questionnaire developed by Block (1989) in assessing intake in low-income black populations. In this case the questionnaire was administered by interview; during this 45 min interview, respondents were asked to recall not only the frequency of consumption and the portion sizes of the ninety-eight food groups included in the questionnaire, but also of any other frequently consumed food. Furthermore, more than half the population studied used vitamin supplements. Intakes of α-carotene, β-carotene, cryptoxanthin and vitamin E were significantly and positively associated with the corresponding serum levels, r values ranging from 0.32 to 0.45 among non-smokers. Among smokers, diet-serum correlations were reduced (r from 0.00 to 0.32).

It is consequently important to consider that our questionnaire has been developed to assess frequency of consumption of food and has not been designed for the assessment of specific micronutrient intake. According to Sempos (1992) the estimates of intake of the nutrients which are not selected as key nutrients in designing a questionnaire may not be accurate. In fact semi-quantitative FFQ are precoded and foods are grouped in order to reduce the number of items listed (sixty-one food groups in ours); consequently the nutrient composition for any food group is an average of the composition for the items within the group. Because of the complexity and variability of nutrient composition of most foods, foods are grouped on the basis of similar composition for only a few nutrients and may give poor estimates of other nutrients. It can sometimes be rather difficult to determine the vitamin intake when for example a subject uses preferentially only one item of the food group and its vitamin content is quite different from the others of the group. For example, our questionnaire had four different groups of cooked vegetables, one including spinach,
beets and other similar vegetables. Unfortunately, even if these vegetables are similar in many ways (including the way of cooking) they are dissimilar for β-carotene content, which is 17640 μg/kg in beets and 47450 μg/kg in spinach. Another example is raw red and yellow vegetables: 1 kg red peppers contains 1400 mg vitamin C, 38400 μg β-carotene and 8000 μg α-tocopherol, while 1 kg tomatoes contains 210 mg vitamin C, 2520 μg β-carotene and 12200 μg α-tocopherol. These differences could help to explain the lack of correlation between dietary intake and plasma values.

For this reason we have now improved the questionnaire, adding the possibility of marking each different vegetable. So far no visual presentation has been provided for this.

When the comparison was made between vitamin levels in plasma and the intake determined from the 7DR the correlation was significant and the classification into tertiles was satisfactory. It has been suggested that correlations in the range of 0.5–0.6 can be considered adequate for studies of the relationship between diet and disease (Block & Hartman, 1989), as they are in the same range as those seen for various physiological measures.

The significant correlation we found when considering the 7DR could depend on the fact that the intake is determined by considering each food separately and, furthermore, the blood is collected during the period of recording; consequently the vitamin content relates to the diet of the days of recording.

In conclusion, the data we obtained provide additional information on the validity of the FFQ method in assessing dietary intake. It seems that frequencies are more useful for defining dietary habits or eating patterns evaluated in terms of macronutrient and mineral consumption, rather than assessing vitamin intake, at least as far as non-supplemented people are concerned. As this study was performed on a group of motivated subjects, further data are needed to extrapolate the results to the general population. Furthermore we would like to focus the attention of researchers on the necessity of having more validation studies on the non-supplemented population, in order to ensure that all the questionnaires proposed are also useful in evaluating vitamin intake in the physiological range of dietary intake.

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REFERENCES


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