Application of a modified Riboflavin load test to a field study of Riboflavin nutritional status in Navajo indians

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SUMMARY

A modified riboflavin load test, consisting of collection of a single two hour urine sample following administration of 1 mg. riboflavin intramuscularly and a simplified fluorometric assay of riboflavin, was studied in 13 male and 51 female Navajo Indian vounteers at the Crown Point Public Health Service Indian Hospital, New Mexico. Average excretion values by this method correlated closely with values by a more complex assay procedure. Average ribofavin excretion values fell within an acceptable range (200 μg. or more) but a disproportionately large number of women under 30 and of post partum women excreted less than 100 μg. of riboflavin in 2 hours.

INTRODUCTION

In the course of a nutrition survey carried out among Navajo Indians of Arizona, Darby *et al.* (1) noted oral and conjunctival lesions suggestive of aribоflavinosis. Dietary data also pointed to a low intake of riboflavin, but no biochemical measurements were available to support these observations.

We have been interested in comparing nutrition status of rural Indian populations with that of Indians relocated in

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urban centers. In the summer of 1965, in order to evaluate laboratory procedures applicable to field studies of riboflavin status, we undertook a preliminary investigation of riboflavin nutritional status among Navajo Indians at the Crown Point Indian Reservation, New Mexico. A modified riboflavin load test with a simplified fluorometric riboflavin assay, suitable for field studies, was compared with results obtained with a more complex assay procedure. An attempt was made to relate urinary riboflavin excretion to riboflavin intake as estimated from dietary recall data.

METHODS

A. *Selection of Subjects*

Thirteen adult male and fifty-one female Navajo Indians were selected for the study, ranging in age from 16 to 77 years. The study group included 15 post partum women tested within two days of admission to the Crown Point Public Health Service Indian Hospital. The other subjects were Indian hospital employees and clinic patients.

B. *Dietary Data*

With the assistance of a Navajo interpreter, a one day recall diet history was obtained from 18 subjects and daily riboflavin intakes were calculated. A one week dietary recall history, limited to eggs, meat, and milk, was obtained from an addtitional 14 subjects. These three foods are the major sources of riboflavin in the Navajo diet. Riboflavin contents of foods were taken from Church and Church (3).

C. *Measurement of Riboflavin Status*

In a preliminary experiment, the oral administration of riboflavin was compared with intramuscular administration in, the same individual, with respect to the pattern of urinary excretion of riboflavin. Urine was collected at 30 minute intervals for 3 hours, and then at 4 hours, following oral intake of 5 mg. riboflavin*. The experiment was repeated, with urine collections at the same intervals, following intramuscular administration of 1 mg. riboflavin. The riboflavin

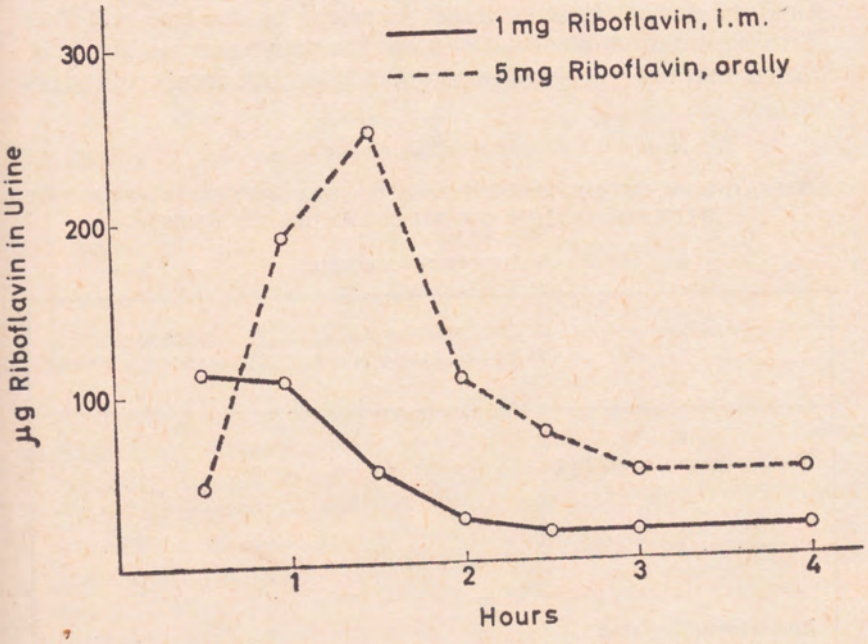
* Hyflavin, Endo Laboratories Inc., Richmond, Hill, California.

content of the urine was estimated by the simplified method, as described.

On the basis of the results of this experiment, a modified Test Dose Excretion Procedure (Lossy, *et al.* 4) was employed. Each subject was given 1 mg. riboflavin by intramuscular injection. A two hour urine sample was collected, following administration of the test dose, and assayed for riboflavin.

A simplified method (I) of urinary riboflavin analysis (5) was employed. The urine sample was diluted so that the riboflavin concentration was approximately 1 μg/ml. The fluorescence was measured in a Turner Fluorometer against a riboflavin standard, using 47-B and 2A-12 filters. Following this, 5 drops of concentrated HCl were added to three milliliters of the sample and the change fluorescence was related to riboflavin concentration, using a standard curve.

FIGURE 1



Riboflavin excretion, in micrograms, following intramuscular administration of 1 mg. riboflavin (————) and following oral administration of of 5 mg. riboflavin (- - - - - -).

Twenty-four urine samples were measured by this method and also by the butanol extraction method of Najjar (II), as modified by Varley (6).

RESULTS

Figure 1 shows riboflavin excretion, in micrograms, following a 5 mg. oral test dose and following a 1 mg. intramuscular test dose. It is seen that the major excretion of riboflavin is completed by 90 minutes after administration of the vitamin. For subsequent studies a 2 hour collection period was chosen for convenience. Use of an oral test dose would have required a longer collection period, but by 3 hours most of the riboflavin excretion had occurred and under conditions where intramuscular injection is not feasible oral administration would appear satisfactory.

Table 1 shows a comparison of the two riboflavin assay methods used. For the 24 individuals studied, the means and standard deviations are almost identical by the two methods. With repeated determinations on the same sample the simplified method (I) gave somewhat lowe but more reproduc-

TABLE 1

COMPARISON OF METHODS I AND II (SEE METHODS) FOR THE DETERMINATION OF RIBOFLAVINE IN URINE

Values in micrograms

	Method I (Simplified Method)	Method II (Extraction Method)
Mean value and standard error (24 samples)	211 ± 27	215 ± 26
Correlation coefficient for methods I and II	0.97	
Reproducibility (ten analyses of a single urine sample)	367 ± 23	413 ± 35

ible results than did th eextraction method (II). The correlation coefficient for the two methods was very high (0.97) and the simplified method was applied to a study of riboflavin status in a sample of the population.

Table 2 shows the average riboflavin excretion (in micrograms) of the 13 Navajo men ad 51 Navajo women. Extrapolating back from the data of Varley (6), an excretion of more than 200 μg. of riboflavin in two hours would be taken as "normal". It is seen that both men and women averaged more than 200 μg. However, although 35 of the 51 women either post partum or were under 30 years of age, these two groups accounted for 13 of the 16 individuals who excreted less than 100 μg. of riboflavin. On the other hand, only 1 of the 13 males excreted less than 100 μg. of riboflavin.

TABLE 2

URINARY RIBOFLAVIN EXCRETION OF NAVAJO INDIANS, FOLLOWING ADMINISTRATION OF 1 mg. RIBOFLAVIN INTRAMUSCULARLY. URINE COLLECTED FOR TWO HOURS. ALL VALUES IN MICROGRAMS. MEANS AND STANDARD DEVIATIONS ARE GIVEN.

Group	Number	Riboflavin Excretion	Range	Number excreting less than 100 mg.
Males	13	272±95	53-396	1
Females	51	221±140	35-516	16

Table 3 shows the average riboflavin intakes, as calculated from 1 day dietary recall records (18 subjects) and from 7 day records of milk, meat, and eggs (14 subjects) and the riboflavin excretion of each group of subjects. The intakes and excretions of each group were compared by calculating the Spearman Rank Correlation Coefficients (7). It is seen that there is an almost complete lack of correlation between dietary data and excretion data.

TABLE 3

CORRELATION OF DIETARY RIBOFLAVIN INTAKE, CALCULATED FROM DIET RECORDS, AND URINARY EXCRETION OF RIBOFLAVIN FOLLOWING INTAMUSCULAR ADMINISTRATION OF 1 mg. RIBOFLAVIN

	One-day diet record (all foods)	Seven-day diet records (milk, meat, eggs)
Number of subjets	18	14
Daily riboflavin intake (average)	545 μg.	450 μg.
Riboflavin excretion (average)	214	288
Correlation coefficient	—0.06	+0.04

DISCUSSION

The results indicate that the simplified procedure used, including collection of a single 2 hour urine sample following intramuscular administration of 1 mg. of riboflavin, and a simplified fluorometric assay for riboflavin, yields useful information on ribloflavin nutritional status and is applicable to field studies under a variety of conditions. Although it requires the use of a fluorometer, the method itself is not complicated or time consuming.

Although the number of subjects studied is small, the results do indicate that a considerable number of young women and post partum women in this population have urinary riboflavin excretions well below that which would be considered desirable. This is in keeping with the observations of Darby *et al* (1) that clinical and dietary signs suggestive of poor riboflavin nutrition occur among Navajo Indians of the Southwestern United States. The results also indicate the desirability of more detailed studies on young women and post partum women in these populations.

In a study of pregnant Guatemalan women of lower economic status, carried out by Arroyave *et al.* (8) a rather similar pattern of riboflavin excretion was found. Of 33 women, with an average 4 hour riboflavin excretion of 251 μg., 6 excreted less than 100 μg. of riboflavin. However, when the Spearman Rank Correlation Coefficient was calculated from their data. A coefficient of +0.87 was obtained, demonstrating a relationship between the dietary intake data and the excretion data.

We suspect that the marked lack of correlation between estimates of dietary riboflavin intake and excretion data in our study is due to our difficulty in obtaining accurate dietary information from this population, even with the assistance of an interpreter. It may be that where a language or communication barrier exist between the investigator and the population under study, biochemical studies offer a more reliable measure of nutritional status than do dietary data.

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RESUMEN

Aplicación de un método modificado de sobrecarga de riboflavina a un estudio de campo sobre el estado nutricional de indios Navajos con respecto a esta vitamina

Una prueba modificada para estudiar riboflavina desde un punto de vista nutricional fue llevada a cabo trabajando con voluntarios indios Navajos (13 varones y 51 hembras) en Crown Point Public Health Service Indian Hospital, New Mexico. Esta prueba consistió en la toma de una sola muestra de orina a las 2 horas después de la administracion intramuscular de 1 mg. de riboflavina. La riboflavina fue determinada fluorométricamente.

Los valores promedio de excreción por este método están muy íntimamente relacionados con los de un ensayo más complejo. Se encontró que los promedios de excreción de riboflavina estaban dentro de un límite aceptable (200 μg. o más), pero se vio que un gran número de mujeres menores de 30 años de edad y mujeres post partum excretaron menos de 100 μg de riboflavina en 2 horas.

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