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A Thiazole Yellow Method for Magnesium Determination in Human Balance Studies

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To the Graduate Council:

I am submitting herewith a thesis written by Evelyn Lorraine Andrews entitled "A Thiazole Yellow Method for Magnesium Determination in Human Balance Studies." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Frances A. Schofield, Major Professor

We have read this thesis and recommend its acceptance:

Bernadine Meyer, Jeannette Biggs

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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ABSTRACT OF EDUCATIONAL RESEARCH STUDY COMPLETED

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Abstract approved by Frances A. Schofield
(signature of major professor)

Note: The student should consult with his major professor and follow his advice concerning the general format of the abstract. Additional pages, if required, should be 8½ x 11 inches and of quality equivalent to that required in the case of the thesis.

The objectives of this work were: to study certain factors affecting the use of a modification of the Young and Gill thiazole yellow colorimetric method for quantitative estimation of magnesium made in the Nutrition Laboratory at the University of Tennessee to permit its use in the analysis of food, feces, and urine; and to compare magnesium determinations obtained by this method with those obtained on the same materials by an established gravimetric procedure. The thiazole yellow method involves the formation of a red magnesium-dye lake, which can be measured colorimetrically, between magnesium hydroxide and thiazole yellow in the presence of a protective colloid. The determination is made directly on the ash solution and interference of other ions is prevented by a compensating solution.

Techniques were devised for preparing completely anhydrous magnesium sulfate to be used in the magnesium standard solution and for weighing the anhydrous salt sufficiently rapidly to keep absorption of moisture at a minimum.

Recoveries of magnesium in standard solutions having a magnesium:calcium ratio of 1:0.7, 1:4, and 1:10 and in fecal ash solutions with ratios of 1:6, 1:7, and 1:16 ranged from 98 to 102%, indicating that the modified compensating solution is effective in preventing interference of calcium over this wide range of concentrations. Interference which occurred in food and fecal samples having a high ratio of total mineral to magnesium was overcome by the use of smaller aliquots or greater dilutions of the test solutions.

Even though the color of the magnesium-dye lake faded with time in blanks, standards, and samples, apparent magnesium concentrations and percentage recovery did not change appreciably when optical density readings were taken at 10, 20, and 30 minutes after addition of dye and base. A time interval of 20 minutes proved most efficient and convenient.

Magnesium determinations on food, feces, and urine by the thiazole yellow method averaged 6, 4, and 6% higher respectively than those by a gravimetric method in which magnesium was precipitated from the calcium-free filtrate as magnesium ammonium phosphate and ignited to magnesium pyrophosphate. This difference was not sufficiently great to prevent comparison of human balance data obtained by the two methods. The fact that the modified thiazole yellow method appears reliable and is rapid seems to justify its use in routine analysis for magnesium in human balance experiments.

August 11, 1960

To the Graduate Council:

I am submitting herewith a thesis written by Evelyn Lorraine Andrews entitled "A Thiazole Yellow Method for Magnesium Determination in Human Balance Studies." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

Frances A. Schofield
Major Professor

We have read this thesis
and recommend its acceptance:

Bernadine Meyer

Jeannette Bigg

Accepted for the Council:

Dean of the Graduate School

A THIAZOLE YELLOW METHOD FOR MAGNESIUM DETERMINATION IN HUMAN BALANCE STUDIES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirement for the Degree

Master of Science

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by

Evelyn Lorraine Andrews

August 1960

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INTRODUCTION

Because a rapid method for the determination of magnesium in food, fecal, and urine composites prepared in human metabolic balance experiments was needed in the Nutrition Laboratory at the University of Tennessee to facilitate analysis of a large number of samples, an attempt was made to apply the thiazole yellow colorimetric method developed by Young and Gill ('51) for analysis of plant tissue. Although the original method did not give reproducible results, a modification was developed suitable for analysis of food composites (Thomas, '56). Further work in this laboratory produced an adaptation of the method which appeared satisfactory for analysis of ash solutions of food, feces, and urine.

The purpose of the work reported in this paper was to study certain factors influencing the use of the modified thiazole yellow colorimetric method for quantitative estimation of magnesium in food, feces, and urine and to compare the results obtained by this method with those obtained by an established gravimetric procedure.

REVIEW OF LITERATURE

The colorimetric determination of magnesium was first suggested by Kolthoff in 1927 when he reported that, in an alkaline solution, magnesium ions change the yellow color of titan yellow and similar dyes to a red color. When sodium hydroxide is added to the solution containing the dye, a red magnesium hydroxide-dye complex is formed that can be measured colorimetrically (Heagy, '48). The intensity of this coloration is related to the concentration of magnesium present (Mitchell, '54). The need for rapid, accurate methods for estimation of magnesium has prompted several investigators to develop methods based upon this principle. Methods have been described for the determination of magnesium in soil extracts, plant tissues, water, and body fluids.

In his review of the use of the thiazole yellow method for determination of magnesium, Mitchell ('54) states that the following facts concerning the method have been established:

1. The same color change that is produced by magnesium is given by other substances under the same conditions.
2. The intensity of the color of the magnesium-dye lake is changed by certain metal ions and

organic compounds.

3. Unless a protective colloid is present, a precipitate rapidly settles from the solution if concentrations of magnesium above three micrograms per milliliter are present.
4. The colored complex is not stable and the intensity of the color decreases with time.

Mikkelsen and Toth ('47) compared different dyes for use in rapid colorimetric methods for estimating magnesium in soil extracts. In this study, thiazole yellow (sodium 2-2 disulfonate of methylbenzolthiazole) was found to be better than titan yellow or Clayton yellow. The color change produced with thiazole yellow was very sharp and clear-cut and its range of sensitivity was broader than that of the other dyes studied.

Effect of Interfering Ions

In modifying a titan yellow method for estimating magnesium in soil extracts, Peech and English ('44) found that serious errors resulted from the interference of ions such as calcium, manganese, aluminum and, to a lesser extent, iron. They stated that in the absence of magnesium, calcium at any concentration failed to give a color lake with titan yellow, but in the presence of magnesium, calcium enhanced or decreased the color of the magnesium-dye lake, depending upon the concentration of magnesium present.

At a low level of magnesium, the presence of 80 times as much calcium as magnesium had no effect on the intensity of the color. At a high level of magnesium, the presence of 5 times as much calcium as magnesium intensified the color. At an even higher level of magnesium, increasing the calcium concentration decreased the intensity of the color of the magnesium-dye lake.

Other workers (Kolthoff, '27; Ludwig and Johnson, '42; Taras, '48; Hirschfelder and Serles, '34) agree that the red color of the magnesium-dye lake is deeper in the presence of the calcium ion, although the ion when present by itself does not produce a color change. In the analysis of soil extracts by a thiazole yellow method, the magnesium-thiazole yellow lake became turbid in the presence of a calcium concentration exceeding 100 times that of magnesium (Mikkelsen and Toth, '47). This interference was overcome by the use of a smaller aliquot of the test solution.

Because of the controversy over whether or not calcium interfered in the determination of magnesium by a titan yellow method, Okamoto and Thomas ('54) compared results of magnesium determinations made directly on bovine serum and on a calcium-free filtrate of the same serum. They obtained two standard curves, one by using standard magnesium solutions with added calcium and one by using standard solutions without added calcium. When readings from the analysis

made directly on the serum were read against the standard curve made with solutions containing calcium, results were similar to those obtained with the calcium-free filtrate read against the standard curve made with solutions not containing calcium. When those made directly on the serum were read against a standard curve not containing calcium, the results were much lower. They concluded that the effect of the amount of calcium present in bovine serum was negligible if calcium was added to blanks and standards.

The amount of calcium in blood or urine did not affect the magnesium determination by a titan yellow method (Orange and Rhein, '51). Removal of calcium ions from solutions of plant tissue when estimating their magnesium content by a thiazole yellow method was unnecessary, but this method did not work with accuracy for the analysis of solutions of lysimeter leachings and rain water unless calcium and R_2O_3 were removed prior to the analysis (Sterges and MacIntire, '50). In the analysis of water (Ludwig and Johnson, '42), accurate determinations were obtained even in the presence of excess calcium in the form of calcium sulfate; it was believed that the electrostatic forces created by the calcium salt and the protective colloid kept the lake in suspension, thus helping to produce readings which were highly reproducible. Gillam ('41) reported that calcium in concentrations up to 800 parts per million did not interfere

with the determination of one part per million of magnesium, but accuracy was improved if the concentration of calcium was below 500 parts per million. In the analysis of body fluids (Kunkel, Pearson, and Schweigert, '47), calcium concentrations below 500 µg per milliliter did not interfere with the color reaction.

Aluminum ion, like calcium, gives no color change with the dyes in alkaline solution but in the presence of magnesium intensifies the color of the magnesium-dye lake (Peech and English, '44; Taras, '48). This effect can be prevented by the addition of aluminum salts to the standards and unknowns in amounts sufficient to produce maximum intensification of the color of the lake.

The effect of manganese is more serious than that of calcium or aluminum because manganese develops a color with thiazole yellow similar to that of magnesium (Young and Gill, '51). This interference may be kept to a minimum by the addition of hydroxylamine hydrochloride and by the addition of manganese to the standards and unknowns. Peech and English ('44) observed that with small concentrations of magnesium, manganese decreased the intensity of the color of the lake or destroyed it; with larger amounts of magnesium, manganese intensified the color of the magnesium lake. It had been reported that hydroxylamine hydrochloride prevented fading of the magnesium lake (Gillam, '41). Peech

and English ('44) felt that the beneficial effect of this reagent was due to its action in preventing manganese interference rather than to any direct effect on the stability of the magnesium-dye lake. They reported an experiment which showed that hydroxylamine hydrochloride did not prevent fading of the magnesium-dye lake in the absence of manganese, but was very effective in preventing manganese interference with the titan yellow test for magnesium. The mechanism for preventing the interference of manganese may involve the formation of a complex and the inhibition of atmospheric oxidation of manganese ion in alkaline solution.

According to Ludwig and Johnson ('42), iron in amounts normally found in natural waters does not seriously interfere with a spectrophotometric determination of magnesium in drinking water; Taras ('48) reported that iron in amounts below 2.5 parts per million did not interfere with a brilliant yellow test for magnesium in water but at higher concentrations intensified the color of the magnesium-dye lake. Orange and Rhein ('51) found that iron did interfere with the titan yellow analysis for magnesium in blood and urine and suggested a method by which iron could be removed effectively with cupferron.

Gillam ('41) and Kunkel *et al.* ('47) agree that the concentration of phosphate ion should be kept below 100 µg per milliliter because above this concentration the color of

the lake is destroyed. This effect occurs because phosphate buffers the solution and lowers the hydroxyl ion concentration. Ammonium ions in concentrations up to 500 or 600 parts per million did not interfere with the color of the magnesium-dye lake (Gillam, '41).

The specific interference of ions seems to be a function of the magnesium concentration of the solution, but the interfering effects vary with different ions. For example, the presence of calcium and aluminum results in abnormally high magnesium values, this effect being greatest at high magnesium levels (Mikkelsen, Toth and Prince, '48).

Elimination of Interference

Several methods have been suggested for elimination of the effects of interfering substances (Mitchell, '54). These methods include removal of interfering ions from solution by precipitation, ignition, or separation as complexes; suppression of their effects by formation of soluble complexes; and standardization of their effects by the use of compensating solutions. The use of compensating solutions is based on the fact that interference of certain ions is relatively constant above specific concentrations (Taras, '48). If fixed amounts of the various ions are added to the test solutions, the effect of the ions already present will be compensated for. When Drosdoff and Nearpass ('48) added to blanks, standards, and samples compensating

solution containing phosphate, calcium, aluminum, and manganese ions at a level 5 times as high as that in the aliquot of ash solution being analyzed, the effect of these ions present in the sample was insignificant. Peech and English ('44) also found that the best way to eliminate calcium and aluminum interference was to add these ions to the standards and unknowns in amounts sufficient to produce maximum intensification of the color of the magnesium-dye lake.

Stability of Color

Several investigators (Gillam, '41; Steyges and MacIntire, '50; Ericsson, '55; Mitchell, '54) have observed that often on standing there is a rapid decrease in the intensity of the red color of the magnesium-dye lake and consistent readings cannot be obtained. Mitchell ('54) believed that this fading is associated with an "ageing" of the magnesium hydroxide. The change responsible for fading starts as soon as the magnesium hydroxide is formed in solution and occurs whether or not the dye is present. He recommended glycerol and a moderate concentration of sodium hydroxide as color stabilizers. The addition of hydroxylamine hydrochloride had no effect. Gillam ('41) stated, to the contrary, that the fading of the color was stopped when a small amount of hydroxylamine hydrochloride was added.

Because of variations in color development, it is necessary to run a standard curve with each series of unknowns (Sterges and MacIntire, '50; Ericsson, '55). In this way, the results of the analysis will not be seriously affected because the change in the color of the unknowns will probably parallel that of the standards.

Protective Colloids

The addition of protective colloids, such as starch, agar, gum ghatti, or polyvinyl alcohol, stabilizes the color by preventing sedimentation of the precipitate (Ericsson, '55). Many protective colloids have been studied, and there has been some controversy over which one is most satisfactory for keeping the dye-lake dispersed. Those studied include starch, dextrin, gums, agar, and polyvinyl alcohol. Hirschfelder and Serles ('34) reported that the addition of soluble starch or dextrin prevented the precipitation of the magnesium-dye lake. Peech and English ('44) found that the intensity of the color of the magnesium lake increased when a protective colloid was used, thus increasing the range and sensitivity of the test. Although they felt that soluble starch and gum tragacanth were equally satisfactory, they preferred starch because gum tragacanth must be purified by electrodialysis. Garner ('46) found that a 1% starch solution lost its protective effectiveness in two or three days and also promoted fading. He discovered that

gum ghatti was superior because it maintained the dispersion for 24 hours or more and in solution was water-clear. Sterges and MacIntire ('50) found that gum ghatti was more satisfactory than gum acacia or starch as a protective colloid.

Orange and Rhein ('51) and Heagy ('48) considered polyvinyl alcohol superior to gum ghatti because it accentuates the color of the dye-lake, thus increasing the sensitivity of the method by at least 15% above that found when gum ghatti is used. In addition, polyvinyl alcohol has the advantage of being a chemical compound which is available in pure form and is easily dissolved. Mitchell ('54), to the contrary, preferred starch because it has no effect on the color of the solution.

Comparison of Colorimetric and Gravimetric Methods

Mikkelsen, Toth and Prince ('48) compared the results of analysis of plant tissues for magnesium by a thiazole yellow method with results obtained by the AOAC gravimetric method in which magnesium is precipitated as magnesium ammonium phosphate and ignited to magnesium pyrophosphate. Average recovery by the thiazole yellow method for 6 samples of bean tissue was 98.8% of that found by the gravimetric method. The range was 94 to 105% recovery. Others also have found good agreement between results of the thiazole yellow method of analysis of plant tissue extracts and the

AOAC gravimetric method (Mitchell, '54; Steiges and MacIntire, '50). Steiges and MacIntire ('50) found that the results from the thiazole yellow method ranged from 0-5% higher than by the gravimetric method. Good agreement between colorimetric analysis of the magnesium content of soil and plant tissue extracts and gravimetric analysis by precipitation of magnesium hydroxyquinolate has been reported (Gillam, '41; Drosdoff and Nearpass, '48).

Excellent agreement has been reported between results obtained by photometric and gravimetric analysis of magnesium in natural water (Ludwig and Johnson, '42; Taras, '48). Analysis of urine by a colorimetric method using titan yellow or Clayton yellow gave results averaging 3% lower than those given by a gravimetric method (Hirschfelder and Serles, '34).

Modification of a Thiazole Yellow Method for Analysis of Food, Feces, and Urine

In the Nutrition Laboratory at the University of Tennessee, an attempt was made to apply the thiazole yellow colorimetric method developed by Young and Gill ('51) for the analysis of plant tissue to the quantitative estimation of magnesium in food composites collected in human balance studies (Thomas, '56). When this procedure was used with food, some solutions became turbid and reproducibility was poor, suggesting that the total mineral content was too high for the dye-lake to be stabilized by the protective colloid.

To reduce the mineral content, calcium was removed from the compensating solution and added to the blanks and standards in approximately the amount known to be in the aliquot of the sample. When this modification was used, turbidity was eliminated, but recoveries were poor. This suggested that the balance between enhancement and suppression of the color of the magnesium-dye lake had been upset by the change in the compensating solution. Reducing the concentration of copper and manganese in the compensating solution and decreasing the concentration of hydroxylamine hydrochloride improved the recoveries and provided a modification suitable for the analysis of ash solutions from food composites. However, this modification was not applicable to ash solutions of fecal samples. Further work in this laboratory produced an adaptation of the method which appeared satisfactory for the analysis of food, feces, and urine. This modification has since been used successfully for routine analysis in this laboratory. The details of the modified method are presented in the section entitled "PROCEDURE."

PROCEDURE

The preliminary part of the work reported here was concerned with factors, such as the presence of other minerals and the time of color development, which influence the use of the modified thiazole yellow colorimetric method for analysis of magnesium in food, feces, and urine. Although the method has been used to analyze samples containing a wide range of magnesium to calcium ratios, more information was needed concerning the range of magnesium:calcium concentrations which was controlled by the compensating solution. Techniques for preparing the anhydrous magnesium sulfate to be used in the standard solution and for weighing the anhydrous salt rapidly in order to keep absorption of moisture at a minimum were devised. The main part of the study dealt with a comparison of magnesium determinations obtained by the modified thiazole yellow colorimetric method with those obtained on the same materials by an established gravimetric procedure.

Materials Analyzed

The materials analyzed in the preliminary part of this study were ash solutions of feces and urine which had been prepared previously in this laboratory and analyzed for calcium, magnesium, and phosphorus. These samples were convenient for the study of the effect of added calcium because

the original calcium content was known. The method under study had been used previously to determine the magnesium content of these materials.

Materials used for direct comparison of the results of the colorimetric method with the gravimetric method were prepared in the following manner. Urine collected over a 24 hour period was pooled and diluted to 1200 ml. Two aliquots of this composite were ashed and solutions prepared for colorimetric analysis. Three aliquots of the same urine composite were analyzed gravimetrically. Ash solutions of food and fecal composites were prepared from frozen samples available from previous experiments. Three aliquots of each of these ash solutions were analyzed for magnesium by the gravimetric method. Two aliquots of the same solutions were diluted and analyzed colorimetrically. Ashing of food and feces was not carried out in duplicate as is routine in the Nutrition Laboratory because the large quantity of composite required to yield sufficient magnesium pyrophosphate for accurate weighing was not available in duplicate amounts. Colorimetric determinations were made on the same solutions of ash because of the need to make a direct comparison of the results of the two methods.

Ash solutions were prepared by weighing aliquots of food (125 gm), feces (130 gm), and urine (50 ml) composites into silica dishes, evaporating to dryness, and ashing at

550°C for 24 hours in the muffle furnace. After cooling, the material was moistened with distilled water and enough concentrated hydrochloric acid was added to dissolve the ash upon heating. The solution was diluted, filtered through ashless filter paper, and made up to a convenient volume for analysis by the colorimetric or gravimetric method.

Effect of Magnesium:Calcium Ratio

In order to determine that the compensating solution prevented interference of calcium over the wide range of magnesium to calcium ratios commonly encountered in the analysis of food, feces, and urine in this laboratory, known amounts of calcium, in the form of calcium chloride solution, were added to solutions of standard containing 15 µg magnesium per aliquot at the following magnesium to calcium ratios: 1:0.7, 1:4, and 1:10. These samples were analyzed as if they were unknowns by the modified thiazole yellow colorimetric method which is described in the next section. The "unknowns" were read against standards and blanks prepared in the usual way. Recovery of magnesium was calculated by dividing the result of the analysis by the amount of magnesium known to be present in the solution analyzed.

An attempt was made to study the effect of large quantities of calcium in fecal ash solutions by adding calcium to fecal solutions previously shown by analysis to

contain 130 µg calcium and 7.7 µg magnesium per 2 ml aliquot. The levels of calcium added were 50 µg, 100 µg, 130 µg, and 150 µg. The fecal solution was also analyzed without any added calcium. When analysis of the solutions containing added calcium proved impossible because of clouding during color development, information concerning the possible influence of the magnesium to calcium ratio was obtained by a study of the recovery of magnesium added to fecal ash solutions of varying calcium and magnesium concentration. Results and recoveries from previous analyses in this laboratory were assembled to aid in this study.

Colorimetric Method of Analysis

The modified thiazole yellow colorimetric method developed and used in this laboratory for determination of magnesium in food, feces, and urine is described in detail below. The procedure and reagents given were used throughout the present study.

The Klett-Summerson photoelectric colorimeter with No. 54 filter was used for reading optical density. Since the readings of this instrument are given on a logarithmic scale, they are proportional to optical density and concentration of color-forming substances and can be used directly in making the standard curve. Colorimeter tubes were matched by selecting tubes which gave readings within

two scale divisions of each other on the same red-colored solution.

The following reagents were prepared in quantity at the beginning of the study and when needed as work progressed.

1. Hydroxylamine hydrochloride, 0.75% (w/v)
2. Compensating solution, grams per liter:

Calcium chloride, ($CaCl_2$)	0.280
Aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$	0.370
Manganous sulfate, $MnSO_4 \cdot H_2O$	0.080
Sodium phosphate, $Na_3PO_4 \cdot 12H_2O$	0.700
Copper sulfate, $CuSO_4 \cdot 5H_2O$	0.0240
Hydrochloric acid, concentrated	5.960 (5 ml)

3. Polyvinyl alcohol, Du Pont, Elvanol, Grade 71-24, 2% (w/v). Twenty grams of polyvinyl alcohol were mixed with about 400 ml of warm distilled water and warmed, with stirring, on a hot plate until dissolved. The solution was diluted to 1 liter and stored in the refrigerator.
4. Mixed reagent. Equal parts of 1, 2, and 3 were mixed together fresh daily.
5. Thiazole yellow dye, General Aniline Works, Inc., Albany, New York. A stock solution containing 0.5% (w/v) of dye in 50% ethyl alcohol was prepared and stored in a dark bottle in the

refrigerator where it keeps indefinitely.

6. Sodium Hydroxide, 10 N.

7. Magnesium standard solution, 1 mg magnesium per ml. $MgSO_4 \cdot 7H_2O$ was dehydrated at 300°C for 7 or more hours until constant weight was obtained. The standard solution was prepared by dissolving 1.2375 gm of the anhydrous $MgSO_4$ in distilled water and made to 250 ml. Because the anhydrous salt takes up moisture rapidly, various techniques for preparing and weighing the anhydrous $MgSO_4$ were compared. These are described in the section on "RESULTS AND DISCUSSION."

The procedure for the colorimetric method was as follows:

1. The day's supply of polyvinyl alcohol was removed from the refrigerator about 2 hours before use.
2. Appropriate volumes of samples and standards were transferred in duplicate with Normax pipettes into colorimeter tubes. Three levels of standard which bracketed the magnesium concentrations of the unknowns were sufficient after the range of proportionality had been established and the techniques mastered.
3. Distilled water was added to bring the volume of

all samples and standards up to 5 ml. Blanks containing 5 ml distilled water were prepared. Sometimes, for convenience, the blanks, standards, and unknowns were pipetted into colorimeter tubes and stoppered with corks on the day previous to analysis.

4. Enough mixed reagent for the day was prepared, mixed carefully, and allowed to stand at least 15 minutes.
5. A 0.02% solution of thiazole yellow was prepared daily by diluting 4 ml of the stock dye solution to 100 ml with distilled water.
6. From a burette, 3 ml of mixed reagent were added to each tube, stirring with a small glass rod flattened horizontally at one end.
7. Beginning with the blanks, 1 ml of 0.02% thiazole yellow was added from a burette to one tube and stirred immediately.
8. Rapidly 2 ml of 10 N NaOH were added with a pipette cut off at the tip. The solution was stirred during the addition of the base and the stop watch started. Steps 7 and 8 were repeated for all tubes in order.
9. After exactly 20 minutes, the colorimeter was set at zero with the blank and the optical density

read in all tubes. Readings were made in the same order and at the same rate as the addition of the dye and sodium hydroxide.

To test precision of the method, recoveries were run with each analysis by adding 1 ml of magnesium sulfate solution containing 10 µg of magnesium to additional aliquots of the samples. Percentage recovery was calculated by subtracting the amount of magnesium in the sample from the amount of magnesium in the recovery and dividing by 10. Whenever possible, samples were analyzed at two dilutions. The presence of large quantities of mineral ash prohibited analysis of solutions of food and feces at the higher concentration.

It was necessary to include standards with each set of determinations because the standard curve varied from day to day. All determinations were made in duplicate.

Gravimetric Method of Analysis

In the gravimetric determination of magnesium in food, feces, and urine, the calcium was first removed by precipitation of the oxalate by the method of the Association of Official Agricultural Chemists ('50). In the case of feces, two and one-half times the specified amount of ammonium oxalate was added because of the high calcium content of the fecal sample supplying adequate amounts of magnesium for analysis.

The calcium-free filtrate was then analyzed for magnesium by a modification of the method of Macy ('42) with reference to the techniques of Willard, Furman, and Bacon ('57). The large amounts of ammonium and oxalate ions present were removed by acidifying the filtrate with concentrated nitric acid and concentrated hydrochloric acid and evaporating to dryness. This process was carried out twice. With urine, the remaining organic matter was removed by heating the residue in the muffle furnace overnight at 550°C. Since ash solutions of food and feces were used, this step was not necessary in their analysis. The residue was dissolved in concentrated hydrochloric acid and distilled water and filtered through ashless filter paper into an unscratched beaker.

The magnesium was precipitated as magnesium ammonium phosphate by the addition of diammonium hydrogen phosphate, followed by the addition of filtered concentrated ammonium hydroxide until the solution was strongly alkaline to methyl red. With food and fecal samples, ammonium tartrate solution was added with the diammonium hydrogen phosphate to keep the iron and aluminum present in solution. After standing in the refrigerator overnight, the precipitate was filtered through a porcelain filtering crucible (previously ignited to constant weight at 1000°C) and washed free of chlorides with dilute, filtered ammonium hydroxide solution.

The precipitate of magnesium ammonium phosphate was ignited to magnesium pyrophosphate by holding at 1000°C for one to one and one-half hours. To prevent cracking, crucibles were placed in the muffle furnace at a temperature of 500°C and the furnace then allowed to heat to 1000°C. After 45 to 60 minutes at this temperature, the furnace was turned off and allowed to cool to 500°C before the crucibles were removed. It was assumed that the crucibles and samples were at constant weight after this period of drying. In preliminary work with the first sample, the crucibles were returned to the furnace and ignited at 1000°C for 30 minutes after being ignited as described above, and the weight remained constant. After cooling in the air for 5 minutes, the crucibles were placed in dessicators and cooled to room temperature before being weighed. The amount of the magnesium present was calculated from the weight of the magnesium pyrophosphate.

All gravimetric analyses were made in triplicate. Recoveries were run by adding a standard solution of magnesium sulfate to two additional aliquots. Percentage recovery was determined by subtracting the average weight of magnesium in the sample from the weight of magnesium in the recovery sample and dividing by the weight of magnesium added. This value was determined by a simultaneous analysis of the volume of standard solution added to the recovery sample.

RESULTS AND DISCUSSION

The work accomplished in the present study consisted of two main problems: evaluation of the influence of certain variables involved in the use of the modified thiazole yellow colorimetric method upon magnesium determinations and a comparison of the results obtained in the analysis of food, fecal, and urine solutions by the colorimetric method and a gravimetric procedure.

Preparation of Magnesium Standard

Since in any colorimetric method of analysis, determinations are based on comparisons with solutions of known concentration, the reliability of methods for the preparation of standard solutions is essential. In the case of magnesium, preparation of standard solutions is complicated by several factors. Magnesium metal is too readily oxidized to permit use of standard wire such as that available for iron and less reactive metals. Furthermore, magnesium salts, such as the phosphates and sulfates, tend to precipitate as mixtures rather than as single chemical compounds. For this reason, the compounds generally preferred for use in standard solutions have been freshly prepared magnesium pyrophosphate obtained by ignition of magnesium ammonium phosphate, or anhydrous magnesium sulfate. The latter is the compound used in the Nutrition Laboratory. Since magnesium

sulfate forms several different hydrates, the anhydrous salt must be freshly prepared and weighed under carefully controlled conditions to provide reliable standard solutions. In the present study, conditions for the preparation of completely anhydrous magnesium sulfate were established and a technique devised for weighing samples rapidly enough to keep absorption of moisture at a minimum.

The first problem in working with the method for preparing the magnesium standard solution was to find the best procedure for obtaining completely anhydrous $MgSO_4$. Samples of $MgSO_4 \cdot 7H_2O$ were weighed into crucibles which had been ignited to constant weight. The hydrate was dried in the furnace at 300°C for 7 hours, then transferred to an oven at 100°C and left overnight. The crucibles were transferred to a desiccator, allowed to cool to room temperature, and weighed. To determine if this method of drying removed all of the moisture, the crucibles were returned to the furnace at 300°C for one hour. When they were weighed again, the weight was less, indicating further loss of water; they were returned to the furnace, weighed, and constant weight obtained. This procedure was repeated and similar results obtained. It is, therefore, recommended that the $MgSO_4 \cdot 7H_2O$ be dried in the muffle furnace at 300°C for 7 or more hours until constant weight is reached to obtain completely anhydrous $MgSO_4$.

The second problem was to devise techniques which would insure a minimum absorption of moisture by the hydrate during weighing. In weighing a sample of 1.2375 gm, the dried magnesium sulfate gained 0.1 mg or more in weight per minute. Thus appreciable errors would be introduced if several minutes were spent in transferring and weighing the magnesium sulfate after it has been dried. So that the time spent in transferring and weighing would be short, the approximate weight of $MgSO_4 \cdot 7H_2O$ needed to obtain the desired amount of $MgSO_4$ was calculated from the empirical formula. This amount was weighed into a weighed, dry crucible and dehydrated as described previously. An additional sample was dried in a second crucible. After constant weight was reached, a small amount of anhydrous $MgSO_4$ was transferred from the second crucible to the first to obtain the desired weight of anhydrous salt. The amount of $MgSO_4$ in the $MgSO_4 \cdot 7H_2O$ in crucible #1 was calculated to be 49.72% and in crucible #2, 49.73%. This procedure was repeated using the average of these two figures to determine the amount of $MgSO_4 \cdot 7H_2O$ required. Again the weight loss slightly exceeded the estimate and a small amount of anhydrous $MgSO_4$ was added from a second crucible to obtain 1.2375 gm.

To make weighing more rapid, a Gram-atic Balance was used. A desiccant was present in the balance case to help

keep the moisture content of the air low.

As a result of this study, it is recommended that the weight of $MgSO_4 \cdot 7H_2O$ needed to obtain the desired amount of anhydrous $MgSO_4$ be calculated from the empirical formula or from the $MgSO_4$ content determined in preliminary trials. This amount, along with an extra amount in a second crucible, should be dehydrated as recommended previously. A small amount of $MgSO_4$ can be transferred rapidly from the second to the first crucible if it is needed. Weighing should be done on a Gram-atic or similar rapid balance. By following this procedure, a minimum amount of time will be spent in transferring and weighing the anhydrous $MgSO_4$.

Effect of Magnesium:Calcium Ratio

The range of calcium concentrations over which the compensating solution prevented interference was studied by adding known amounts of calcium to standard solutions of magnesium sulfate and to aliquots of solutions of ash from fecal composites.

Mention has been made previously of the relatively high mineral concentration in ash solutions of feces. In the feces of individuals on normal human diets, the amount of magnesium present is likely to be low in comparison to the total mineral content. For these reasons analysis of ash solutions of fecal composites by the modified thiazole yellow method has required special precautions. Work with

a fecal composite having the smallest magnesium:calcium ratio encountered in studies in this laboratory, 1:16, illustrates the difficulties. When the solution of ash was diluted to give a magnesium ion concentration of approximately 8 µg of magnesium per 2 ml aliquot, analysis appeared satisfactory. Addition of 10 µg of magnesium as standard solution to 2 ml of this dilute solution had no adverse effect upon the analysis. However, when 50 µg of calcium were added, increasing the calcium ion concentration from 130 to 180 µg per aliquot, cloudiness was noticed during color development and readings were high. Addition of 100 µg or more of calcium caused immediate precipitation when base was added. Whether this interference was due to calcium ion alone or to an increase in cation concentration above the ability of the protective colloid to prevent coagulation cannot be stated with certainty. In the analysis of other ash solutions of food and feces in the present study, however, similar difficulty was encountered when attempts were made to carry out analyses at two different dilutions. At the greater dilution, the solutions were clear and results were reproducible on different days, but at the higher concentration, the solutions became cloudy and results were high. This interference could be overcome by the use of a smaller aliquot of the test solution as suggested by Mikkelsen and Toth ('47). For the food and feces samples being

analyzed in this study, it was necessary to analyze samples with a magnesium content of 5 to 10 µg because the ratio of total minerals to magnesium was apparently high. In previous work in this laboratory, it was possible to analyze food and fecal samples at higher levels of magnesium (table 1) when the ratio of total ash to magnesium was lower. Urine samples were satisfactorily analyzed at concentrations of magnesium up to 30 µg per aliquot because the ash content was low in relation to the magnesium content.

In work done in this laboratory, the range of magnesium to calcium ion concentrations in the materials under study has been as follows: urine, 1:0.5 to 1:2; feces, 1:5 to 1:16; food, 1:4 to 1:7. The influence of the relative amounts of calcium upon analysis was studied through determination of the percentage recovery of added magnesium in standard solutions having a magnesium:calcium ratio of 1:0.7, 1:4, and 1:10 and in fecal ash solutions in which previous analysis indicated ratios of 1:6, 1:7, and 1:16. As shown in table 1, recoveries of added magnesium for the three magnesium:calcium ratios in the standard solutions were 100, 98, and 100%, indicating that the compensating solution is adequate over this wide range when no other mineral is present. Of even greater importance is the fact that recoveries with fecal solutions, containing undetermined concentrations of other minerals, were also excellent

TABLE 1

INFLUENCE OF MAGNESIUM:CALCIUM RATIO ON PERCENTAGE RECOVERY OF ADDED MAGNESIUM

Sample	Magnesium/ aliquot (µg)	Mg:Ca	1	2	3	Av.
Standard Solution						
	15	1:0.7	102	101	98	100
	15	1:4	99	98	--	98
	15	1:10	98	105	97	100
Fecal Ash Solution						
#5[a]	11.4	1:6	101	100	--	100
#6[a]	18.4	1:6	97	100	--	98
#7[a]	10.6	1:7	98	101	--	100
#8[a]	18.7	1:7	102	103	--	102
#9	7.7	1:16	102	--	--	102

[a]Results from previous analyses in this laboratory.

(98 to 102%) at different magnesium:calcium ratios. Recoveries were about the same at the 1:16 ratio as at the 1:6 ratio, 102 and 100% respectively. Recoveries of added magnesium were equally as good at magnesium:calcium ratios ranging from 1:0.7 to 1:16 suggesting that the compensating solution is effective in preventing interference over this wide range.

Effect of Time

Several workers have observed, when using colorimetric methods, that the intensity of the color of the magnesium-dye lake often decreases on standing (Gillam, '41; Sterges and MacIntire, '50; Eriesson, '55; Mitchell, '54). In the present study, this fading was noted also. Optical density readings were made 10 minutes after the addition of the dye and base in the original method (Young and Gill, '51). Because it seemed more economical of time to be able to include more samples in a single series of determinations than was possible when readings were made after 10 minutes, readings and apparent magnesium concentrations of the same solutions were checked at 10, 20, and 30 minutes after the addition of the dye and sodium hydroxide to see if the time interval changed the results. In most cases, there was a steady fading of the color of the magnesium-dye lake from 10 to 30 minutes in the blanks, standards, samples, and recoveries. The colorimeter was set at zero with the blank

at the start of each series of readings, the routine procedure in analysis by the thiazole yellow method. A typical series of comparisons is presented in table 2. The value of the colorimeter reading decreased with each time interval, reflecting a diminishing of the intensity of color of the magnesium-dye lake. The rate of fading varied from day to day and occasionally the reading at 20 minutes was higher than at 10 minutes, but it was always lower at 30 minutes than at 20 minutes. The rate of fading also varied from sample to sample in the same series. Mitchell ('54) felt that this fading was associated with an "ageing" of the magnesium hydroxide which occurred whether or not the dye was present.

The results of the analysis of samples did not change appreciably from one time interval to the next (table 3, page 34), probably because the color produced with standards and samples faded at about the same rate (table 2). Sterges and MacIntire ('50) also felt that fading did not interfere with the analysis if at least three known standards were run with each series of samples and if each series was read after the same time interval.

Recoveries were run by adding known amounts of magnesium to duplicate aliquots of sample. In some instances, as with urine #2 (table 3), recovery improved somewhat from 10 minutes to 30 minutes while the actual analysis remained

TABLE 2

EFFECT OF TIME ON INTENSITY OF COLOR OF THE MAGNESIUM-DYE LAKE

Sample	Time Interval (min)	Colorimeter Reading[a]
Standard Solution		
10 µg Magnesium	10	79
	20	78
	30	75
20 µg Magnesium	10	148
	20	146
	30	142
30 µg Magnesium	10	219
	20	215
	30	210
Solution of Ash		
Food #3	10	85
	20	83
	30	81
Food #3 plus 10 µg Magnesium	10	155
	20	154
	30	150
Urine #1	10	144
	20	143
	30	141

[a]Average of readings from duplicate tubes.

TABLE 3

EFFECT OF TIME INTERVAL ON ANALYSIS OF SOLUTIONS

Sample	Time Interval (min)	Analysis (µg Mg per ml)	Recovery of Added Mg (%)
Food #3	10	11.3	93
	20	11.2	96
	30	11.2	96
Feces #1	10	5.7	99
	20	6.3	98
	30	6.1	96
Feces #2	10	7.2	97
	20	7.2	99
	30	7.3	98
Urine #2	10	8.8	92
	20	8.9	95
	30	8.9	97
Urine #3	10	17.0	96
	20	17.1	97
	30	17.5	98

the same. This example points out the uneven fading which occurred from sample to sample. In other instances (feces #1) there was a slight decrease in per cent recovery with increase in time. The fact that there was no consistent trend in recovery of added magnesium and that the results of the analysis did not change appreciably when readings were made between 10 and 30 minutes following color development indicates that results obtained at any time within this interval are equally reliable. As long as all readings in a series are made at the same time interval after color development, the fading will not affect the analysis.

A time interval of 20 minutes between the addition of the dye and base and the reading of the optical density has proved convenient in this laboratory. Color can be developed and readings made on 18 to 20 pairs of tubes in 40 minutes. Reading the optical density at 10 minutes after the addition of dye and base seems inefficient since three standards and a blank must be included in duplicate with each series; only 5 or 6 samples can be analyzed as compared with 14 or 16 when readings are made at 20 minutes. Making readings at 20 minutes, rather than 10, has the additional advantage that most of the bubbles formed by addition of the polyvinyl alcohol have escaped during the longer interval. Disadvantages of making readings at 30 minutes after addition of dye and base include the danger of error from

fatigue, when color must be developed in a large number of tubes at a constant rate, and the possible formation of turbidity in solutions of high mineral content.

Comparison with a Gravimetric Method

Since, until recently, most of the data from magnesium balance studies in humans have been obtained by use of a gravimetric method, in which magnesium is precipitated from the calcium-free filtrate as magnesium ammonium phosphate and ignited to magnesium pyrophosphate (Daniels and Everson, '36; Bogert and McKettrick, '22; Chaney and Blunt, '25; Hummel et al., '36; Hummel et al., '37; Macy, '42), comparison of the results obtained by the modified thiazole yellow method with analyses made by the gravimetric procedure appeared essential. To make this comparison, aliquots of the same composites of food, feces, and urine were analyzed by both methods.

The results of analyses by the colorimetric method are shown in table 4. The levels of magnesium per 1 ml aliquot of ash solution and duplicate ash solution are presented to show the reproducibility of analyses by the modified thiazole yellow method. Determinations of recovery of magnesium added to other aliquots of the same solutions of ash are included to indicate the accuracy obtainable and the effectiveness of the compensating solution in the control of interference by other ions. The magnesium

TABLE 4

COLORIMETRIC DETERMINATION OF MAGNESIUM

Sample	Solution of Ash				Composite	
	Analysis		Recovery Added Mg			
	1	2	1	2	1	2
Food						
	µg per ml		%		mg per 100 gm	
#1	14.6	14.1	102	99	20.8	20.1
Average	14.4		100		20.4	
#2	6.2	6.0	91	--	24.8	24.0
Average	6.1		91		24.4	
#3	11.4	10.6	96	97	22.8	21.2
Average	11.0		96		22.0	
Feces						
	µg per ml		%		mg per 100 gm	
#2	7.2	7.4	94	97	27.6	28.5
Duplicate	7.5	7.7	100	99	28.8	29.6
Average	7.4		98		28.6	
#3	6.7	6.8	95	94	25.8	26.2
Duplicate	6.8	6.8	98	94	26.2	26.2
Average	6.8		95		26.2	
#4[a]	10.8	10.2	96	99	20.8	19.6
Duplicate[b]	8.1	8.1	102	103	20.8	20.8
Average	--		100		20.5	
Urine						
	µg per ml		%		mg per 24 hr	
#1	9.0	9.2	98	94	108.0	110.4
Duplicate	9.2	8.9	--	--	110.0	106.8
Average	9.1		96		108.9	
#2	15.7	15.5	94	98	94.2	93.0
Duplicate	15.7	15.4	--	99	94.2	92.4
Average	15.6		97		93.4	

[a] 3:50 dilution

[b] 1:25 dilution

concentration per 100 gm of food and fecal composite and per 24 hour urine sample are included for comparison with the analyses made by the gravimetric method.

Reproducibility between the two analyses of the same sample and between duplicates of food, feces, and urine was good (table 4), indicating satisfactory precision of the method. Average deviation of individual analyses from the mean was 3% for food, 2% for feces, and 1% for urine.

Recoveries ranged, for the most part, from 95 to 103%, indicating that the thiazole yellow method is accurate for use in analysis of food, feces, and urine. To show that recoveries with food solutions are usually equally as good as with urine and feces, results of the colorimetric analysis of food #1 are included in table 4, even though gravimetric analysis of this sample was not made. Recoveries averaged nearly the same for the three biological materials: 97% for food, 98% for feces, and 97% for urine. Thus, in human balance studies, the data obtained in the quantitative estimation of magnesium in all three types of biological materials by the thiazole yellow method should be comparable. Accuracy, as indicated by recoveries, was equally good with urine #2 containing 15.6 µg magnesium per ml and with feces #2 containing about half as much magnesium, indicating that the compensating solution effectively prevented the interference of other ions present in the samples analyzed.

The magnesium content of the food, fecal, and urine samples as estimated quantitatively by the gravimetric method is shown in table 5. In the case of food and feces, two or three aliquots of the same ash solution were analyzed and the results calculated on the basis of mg of magnesium per 100 gm of composite. With urine, two or three samples of the composite were analyzed and the results given in mg of magnesium per 24 hour output. Recoveries of added magnesium are included also to indicate the accuracy obtained.

Precision by this procedure was good as indicated by the close agreement between replicate analyses of the sample (table 5). Average deviation of individual analyses from the mean was 0% for food, 1% for feces, and 2% for urine. Accuracy, as indicated by average per cent recovery, was good, ranging from 92 to 103%. The recoveries averaged about the same in the analysis of food and feces, 98 and 100% respectively. The average recovery for urine was somewhat lower, but this was probably due to the greater possibility for error present during the analysis; a large volume of liquid was evaporated to dryness in a silica evaporating dish and spattering was a constant possibility.

The results obtained by the colorimetric method and the gravimetric method are summarized in table 6, page 41. Results of the analysis of food, feces, and urine for magnesium by the colorimetric method were consistently higher

TABLE 5

GRAVIMETRIC DETERMINATION OF MAGNESIUM

Sample	Replicate Analysis				Replicate Recovery of Added Magnesium		
	1	2	3	Av.	1	2	Av.
			Food				
		mg per 100 gm			% Recovery		
#2	22.4	22.4	22.4	22.4	94	96	95
#3	21.6	21.6	--	21.6	102	98	100
			Feces				
		mg per 100 gm			% Recovery		
#2	27.2	28.0	27.6	27.6	96	96	96
#3	24.5	24.5	25.0	24.7	102	100	101
#4	19.7	20.2	20.2	20.0	101	104	103
			Urine				
		mg per 24 hr			% Recovery		
#1	106.2	102.0	--	104.1	92	--	92
#2	87.6	86.4	82.8	85.6	98	89	94

TABLE 6

COMPARISON OF MAGNESIUM CONTENT OF FOOD, FECES, AND URINE SAMPLES AS DETERMINED BY TWO METHODS

Sample	Gravimetric Method	Colorimetric Method	Difference
		Food	
		mg per 100 gm	%
#2	22.4	24.4	9
#3	21.6	22.0	2
		Feces	
		mg per 100 gm	%
#2	27.6	28.6	4
#3	24.5	26.2	7
#4	20.2	20.5	2
		Urine	
		mg per 24 hr	%
#1	104.1	108.9	4
#2	85.6	93.4	9

than those by the gravimetric method. With food, the results by the colorimetric method ranged from 2 to 9% higher, with feces, the range was 2 to 7% higher, and with urine, the range was 4 to 9% higher. The difference between the results of the analysis of urine as obtained by the two methods in this study does not agree with that found by Hirschfelder and Serles ('34). They report that results obtained when determining the magnesium content of urine with a colorimetric method using titan yellow or Clayton yellow averaged 3% lower than results obtained using a gravimetric procedure. No other comparisons of colorimetric and gravimetric methods using food, feces, or urine were found in the literature, but several comparisons have been made using other materials. For example, Sterges and MacIntire ('50) found that analysis of plant tissue by the thiazole yellow method gave results from 0 to 5% higher than the AOAC gravimetric method.

Comparison of the data obtained in the analysis of composites of food, feces, and urine for magnesium by the University of Tennessee modification of the thiazole yellow colorimetric method and by the older gravimetric techniques indicated that the limits of experimental error, as shown by recovery of added magnesium, of the two methods were similar (table 4 and table 5). Analyses by the colorimetric method were consistently higher than those obtained by the

older method. However, since the differences were of the same magnitude for all three types of biological material, balance data obtained by the two methods should be entirely comparable. In no case was the difference in the estimated magnesium content of food, feces, or urine sufficiently great to indicate a real difference in dietary level of magnesium or in its metabolism in a comparison of human balance data obtained by the two methods. Since the comparison of analyses by the two procedures indicated that the data obtained by the colorimetric method are no less reliable than those obtained in the gravimetric procedure and since the rapidity of the colorimetric method has the obvious advantage of facilitating the study of magnesium metabolism and requirements in humans, the adoption of the modified thiazole yellow method for routine use appears justified.

SUMMARY

In work reported in this paper, several factors affecting the University of Tennessee modification of a thiazole yellow colorimetric method for determination of magnesium in human balance experiments were studied. Comparison of analyses by this method was made with those from a gravimetric method used in obtaining much of the data on magnesium balances reported in the literature.

Conditions for preparing completely anhydrous magnesium sulfate were established and a technique devised for weighing samples rapidly in order to keep absorption of moisture at a minimum.

The influence of magnesium:calcium ratios was studied through determination of the percentage recovery of magnesium in standard solutions having a magnesium:calcium ratio of 1:0.7, 1:4, and 1:10 and in fecal ash solutions with ratios of 1:6, 1:7, and 1:16. Recoveries ranged from 98 to 102% indicating that the modified compensating solution is effective in preventing interference of calcium over this wide range. Solutions became turbid after addition of dye and base when the level of calcium was increased in a fecal solution with a magnesium:calcium ratio of 1:16. Solutions also became turbid during the analysis of food and fecal samples which had a high ratio of total mineral to magnesium. This interference was overcome by the use of

smaller aliquots or greater dilutions of the test solution.

Apparent magnesium concentration and percentage recovery of added magnesium did not change appreciably when optical density readings were taken at 10, 20, and 30 minutes after addition of dye and base during analysis of food, fecal, and urine samples, even though there was a fading of the color of the magnesium-dye lake in the blanks, standards, and samples with passage of time. A time interval of 20 minutes has proved most efficient and convenient in the Nutrition Laboratory.

Magnesium determinations on food, feces, and urine by the thiazole yellow method were consistently higher than by a gravimetric procedure. However, in no case was the difference sufficiently great to prevent comparison of human balance data obtained by the two methods. The fact that the modified thiazole yellow method appears reliable and is rapid seems to justify its use in routine analysis for magnesium in human balance studies.

BIBLIOGRAPHY

BIBLIOGRAPHY

Association of Official Agricultural Chemists 1950 Official Methods of Analysis, ed. 7, Washington, D. C.

Bogert, L. J. and E. J. McKettrick 1922 Studies in inorganic metabolism. I. Interrelations between calcium and magnesium metabolism. J. Biol. Chem., 54:363.

Chaney, M. S. and K. Blunt 1925 The effect of orange juice on the calcium, phophorus, magnesium and nitrogen retention and urinary organic acids of growing children. Ibid., 66:829.

Daniels, A. L. and G. L. Everson 1936 A study of the magnesium needs of preschool children. J. Nutrition, 11:327.

Drosdoff, M. and D. C. Nearpass 1948 Quantitative microdetermination of magnesium in plant tissue and soil extracts. A rapid colorimetric method. Anal. Chem., 20:673.

Ericsson, Y. 1955 Simplified methods for determination of calcium and magnesium in the saliva. J. Dental Research, 34:104.

Garner, R. J. 1946 Colorimetric determination of magnesium in plasma or serum by means of titan yellow. Biochem. J., 40:828.

Gillam, W. S. 1941 A photometric method for the determination of magnesium. Ind. Eng. Chem., Anal. Ed., 13:499.

Heagy, F. C. 1948 The use of polyvinyl alcohol in the colorimetric determination of magnesium in plasma or serum by means of titan yellow. Can. J. Research, 26E:295.

Hirschfelder, A. D. and E. R. Serles 1934 A simple adaptation of Kolthoff's colorimetric method for the determination of magnesium in biological fluids. J. Biol. Chem., 104:635.

Hummel, F. C., H. A. Hunscher, M. F. Bates, P. Bonner and I. Macy 1937 A consideration of the nutritive state in the metabolism of women during pregnancy. J. Nutrition, 13:263.

Hummel, F. C., H. R. Steinberger, H. A. Hunscher and I. G. Macy 1936 Metabolism of women during the reproductive cycle. Ibid., 11:235.

Koltheff, I. M. 1927 Specific colour reaction for magnesium and a colorimetric method for determination of traces of magnesium. Analyst, 52:430.

Kunkel, H. O., P. B. Pearson and B. S. Schweigert 1947 The photoelectric determination of magnesium in body fluids. J. Lab. Clin. Med., 32:1027.

Ludwig, E. E. and C. R. Johnson 1942 Spectrophotometric determination of magnesium by titan yellow. Ind. Eng. Chem., Anal. Ed., 14:895.

Macy, I. G. 1942 Nutrition and Chemical Growth in Childhood. Vol. I. Evaluation. Charles C. Thomas, Springfield, Ill.

Mikkelsen, D. S. and S. J. Toth 1947 Thiazol yellow for determining the magnesium content of soil extracts. J. Am. Soc. Agron., 39:165.

Mikkelsen, D. S., S. J. Toth and A. L. Prince 1948 Determination of magnesium by thiazol yellow method. Soil Science, 66:385.

Mitchell, T. A. 1954 The spectophotometric determination of magnesium with thiazol yellow dyes. Analyst, 79:280.

Okamoto, Mitsuo and J. W. Thomas 1954 Magnesium determination in bovine blood serum. Anal. Chem., 26:1072.

Orange, M. and H. O. Rhein 1951 Microestimation of magnesium in body fluids. J. Biol. Chem., 189:379.

Peech, M. and L. English 1944 Rapid microchemical soil tests. Soil Science, 57:167.

Sterges, A. J. and W. H. MacIntire 1950 Magnesium content of plant tissue. Microchemical determination through thiazole yellow procedure. Anal. Chem., 22:351.

Taras, M. 1948 Photometric determination of magnesium in water with brilliant yellow. Ibid., 20:1156.

Thomas, H. J. 1956 A modification of the thiazole yellow colorimetric method for determination of magnesium in selected biological material. Master's thesis. University of Tennessee.

Willard, H. H., N. H. Furman and E. K. Bacon 1957 A Short Course in Quantitative Analysis. D. Van Nostrand Co., Inc., Princeton, New Jersey.

Young, H. Y. and R. F. Gill 1951 Determination of magnesium in plant tissue with thiazole yellow. Anal. Chem., 23:751.