through a soil saturated with nitrifying bacteria is evidence that X must first be converted by other (non-stimulated) organisms into NH_4^+ which can then be attacked by the nitrifiers. On the other hand, if the substance under investigation is converted at an immediate linear rate into NO_3^- this is evidence that it may be attacked directly by the nitrifying organisms.

2. Although methylamine and trimethylamine are nitrified by a bacteria-saturated soil, their rates of transformation into NO_3^- show initial time lags which indicate that they are attacked by organisms other than the nitrifiers before nitrification. Tetramethylammonium chloride is nitrified with difficulty; its rate of conversion to NO_3^- shows a large time lag.

3. Urea and glycine are rapidly nitrified by bacteria-saturated soils but their courses of transformation to NO_3^- are not quite linear. Presumably they are attacked by organisms other than nitrifiers before nitrification takes place, or are converted

chemically into substances which the nitrifiers can attack.

4. Guanidine carbonate (0.005 M) is nitrified with difficulty by a bacteria-saturated soil. It inhibits the conversion of NH_4^+ to NO_3^- in soil.

5. Hydroxylamine (0.005 M) is not nitrified by a bacteria-saturated soil. It suppresses the conversion of NH_4^+ to NO_3^- in soil.

6. Hydrazine (0.0025 M) undergoes feeble nitrification in a bacteria-saturated soil and greatly inhibits the conversion of NH_4^+ to NO_3^- in soil.

7. Pyruvic-oxime (0.005 M) is nitrified with great rapidity by a bacteria-saturated soil and does not inhibit the conversion of added NH_4^+ to NO_3^- .

8. The presence of sodium pyruvate (0.005 M) entirely eliminates the toxic effect of hydroxylamine (0.005 M) on soil nitrification and allows full nitrification of the hydroxylamine to take place. The significance of this fact and of the result with pyruvic oxime are briefly discussed.

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Colorimetric Determination of Magnesium in Plasma or Serum by Means of Titan Yellow

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The principal methods employed for the estimation of blood magnesium require the precipitation of the magnesium either as the ammonium phosphate or as the 8-hydroxy-quinolinolate after preliminary removal of calcium by precipitation as oxalate. Of necessity these methods involve several steps, and are cumbersome and lengthy.

Several workers have described methods for the determination of magnesium based on the observation by Kolthoff (1927 *a, b*) that when magnesium is precipitated as the hydroxide by addition of sodium hydroxide in the presence of an acridine-sulpho-dye, Titan yellow, the colour of the dye is changed from yellow to red. Becka (1931) and Hirschfelder & Serles (1934) adapted the method for use with biological fluids, while the method has also found some favour for the determination of mag-

nesium in water (Urbach & Baril, 1934; Müller-Neuglück, 1941; Ludwig & Johnson, 1942, and others).

The methods described for plasma (Becka; Hirschfelder & Serles) are open to some criticism in that these workers did not precipitate the plasma proteins. As the colour of normal plasma may vary from pale yellow to a frank red according to the degree of haemolysis, a considerable error may be introduced. There is also some controversy as to whether the presence of calcium interferes with the determination of magnesium.

In view of these points it was decided to investigate the method and, if possible, to develop a procedure suitable for the determination of magnesium in plasma and serum with the photoelectric colorimeter.

In the method to be described the plasma or serum proteins are precipitated by means of trichloroacetic acid, Titan yellow solution is added to the resulting water-clear filtrate and the magnesium precipitated by addition of sodium hydroxide. Gum ghatti is used to maintain dispersion of the magnesium-dye lake.

METHOD

Solutions

Standard magnesium solutions

Stock standard. 10.131 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ are dissolved in distilled water, 0.5 ml. chloroform added and the volume made to 1 l. with distilled water (1 ml. stock standard contains 1 mg. Mg).

Working standard. 2 ml. of the stock standard are diluted to 100 ml. (1 ml. working standard contains 0.02 mg. Mg).

Trichloroacetic acid. 10% (w/v).

Gum ghatti solution. 1 g. gum ghatti is tied loosely in muslin and left suspended in 1 l. of distilled water overnight. The soluble part of the gum leaches out into the water. The residue is discarded and the solution preserved with a few drops of chloroform.

Titan yellow solution. 0.05%. 100 mg. dissolved in water, made to 200 ml. and filtered. The solution should be kept in a brown bottle and should be freshly prepared about every fortnight.

Sodium hydroxide. 4 N.

are well mixed, allowed to stand for 5 min. and then filtered through a 9 cm. Whatman no. 42 paper. To 8 ml. of the water-clear filtrate 1 ml. of gum ghatti, 1.5 ml. Titan yellow and 2 ml. 4 N-NaOH are added in that order.

A water-blank is prepared by taking 6 ml. distilled water, adding 2 ml. 10% trichloroacetic acid, 1 ml. gum ghatti, 1.5 ml. Titan yellow and 2 ml. NaOH.

A suitable standard for the average range of blood magnesium values is obtained by taking 2 ml. of the working standard solution (equivalent to 2 mg. Mg/100 ml.), adding 4 ml. water, 2 ml. trichloroacetic acid, 1 ml. gum ghatti, 1.5 ml. Titan yellow and 2 ml. NaOH.

The solutions are compared in the photoelectric colorimeter with a green light-filter. The minimum transmittance of the magnesium-dye lake is at 5250 Å. (Fig. 1). The colours remain stable for at least 3 hr.

RESULTS

A calibration curve of the colour produced plotted against varying amounts of a standard magnesium solution (Fig. 2) showed that Beer's law is strictly

Fig. 1. Absorption spectra of the magnesium-Titan yellow lake with varying quantities of magnesium. (Spekker absorptiometer, 1 cm. cells.)

Procedure

To 4 ml. plasma or serum are added 8 ml. water and 4 ml. 10% trichloroacetic acid. The solutions

Fig. 2. Calibration curve obtained with standard magnesium solution. (Spekker absorptiometer, Ilford Spectrum Green 604 filter, 1 cm. cells.)

obeyed over the range 0–0.12 mg. magnesium (equivalent to 0–0.6 mg. Mg/100 ml. original sample).

A series of recoveries was carried out by addition of a known quantity of magnesium to sera of known magnesium content before precipitation of the serum proteins. The results are shown in Table 1.

Table 1. *Recoveries of magnesium added to plasmas and sera of known magnesium content*

(All values are in mg./100 ml.)

	Plasma	Serum
No. of specimens	12	12
Initial magnesium content	0.73-2.63	1.08-2.57
Magnesium added	0.20-2.00	0.20-2.00
Magnesium recovered	0.19-2.05	0.24-1.97
Mean deviation	(Added - Recovered) Nil	+0.01
Standard deviation	0.04	0.03

Table 2. *Effect of added oxalate on the determination of magnesium with Titan yellow*

Photometric readings (Ilford Spectrum Green 604 filter, 1 cm. cell).

Magnesium (mg.)	No oxalate (E)	1 ml. 4% ammonium oxalate (E)	0.1 ml. 30% potassium oxalate (E)
0.00	0.33	0.33	0.33
0.02	0.47	0.47	0.48
0.04	0.60	0.60	—
0.06	0.74	0.74	0.74

A second series of recoveries was made on plasma, after first ascertaining that the presence of oxalate either as 4% ammonium oxalate (as used in the determination of calcium) or as 30% potassium oxalate (used as an anticoagulant for the preparation of plasma) did not interfere (Table 2). The results of the recoveries are shown in Table 1. In both series

Table 3. *Effect of added calcium on the determination of magnesium with Titan yellow using starch and gum ghatti to maintain dispersion of the magnesium-dye lake*

Photometric readings (Ilford Spectrum Green 604 filter, 1 cm. cell).

Magnesium present (mg.)	Amount of calcium added			
	0 (E)	0.05 mg. (E)	0.1 mg. (E)	2.9 mg. (2 ml. sat. calcium sulphate soln.) (E)
Starch as protective colloid				
0.00	0.32	0.33	0.32	0.40
0.02	0.45	0.45	0.45	0.43
0.04	0.59	0.59	0.59	0.55
0.06	0.72	0.71	0.72	0.68
Gum ghatti as protective colloid				
0.00	0.33	0.33	0.33	0.33
0.02	0.48	0.48	0.48	0.49
0.04	0.64	0.64	0.64	—
0.06	0.81	0.82	0.81	—

of recoveries the error falls within the range usually expected in colorimetric determinations.

The effect of calcium on the colour development is shown in Table 3.

DISCUSSION

Kolthoff (1927*a*) and Ludwig & Johnson (1942) stated that the presence of calcium deepened the red colour due to the magnesium-dye lake, giving a minimum transmittance at 5250 Å. Hirschfelder & Series (1934), criticizing the work of Becka (1931), stated that if calcium was not removed an 80% error was introduced. Investigation has shown that the presence of calcium in concentrations of the order usually found in blood (10 mg./100 ml.) does not affect the colour produced when read at 5200 Å. (Ilford Spectrum Green 604 filter) (Table 3).

Fig. 3. Calibration curves obtained with standard magnesium solution using varying amounts of Titan yellow. (Spekker absorptometer, Ilford Spectrum Green 604 filter, 1 cm. cells.)

The accepted medium for maintaining the lake in a state of dispersion has hitherto been starch, other protective colloids having been rejected either because of their translucence or because they were found to contain magnesium. A 1% starch solution was found to lose its protective effect in from 2 to 3 days and also promoted fading. It was therefore decided to replace it by gum ghatti. This maintains dispersion for over 24 hr. and has the additional

advantage of being water-clear and of 'spreading' the readings slightly (Table 3). Contrary to the findings of Hirschfelder & Serles (1934) the sample of gum ghatti used contained no magnesium.

Haury (1937) has said of the direct method of estimation of the magnesium-dye lake 'with this method it is necessary to add an excess of Titan yellow. A definite percentage of the dye combines with or absorbs the magnesium ion to produce the characteristic red magnesium-dye lake. The surplus yellow due remaining in the solution vitiates the red colour to be compared. Consequently unless the concentration of magnesium in the two solutions to be compared be within close range, satisfactory colour matches cannot be obtained.' By choosing a suitable concentration of Titan yellow a very satisfactory adherence to Beer's law can be obtained. The optimum amount was found to be 1.5 ml. of a 0.05% solution for the range over which blood magnesium may be expected to vary. The effect of less or more Titan yellow is shown in Fig. 3.

The amount of sodium hydroxide added must be sufficient to neutralize the excess trichloroacetic acid remaining after precipitation of proteins and to precipitate the magnesium hydroxide. 2 ml. of a 4N-solution was found to provide ample excess.

SUMMARY

1. A rapid and accurate method is described for the estimation of magnesium in plasma or serum depending upon the precipitation of magnesium hydroxide by sodium hydroxide in the presence of Titan yellow.

2. The estimation may be carried out in the presence of calcium or of oxalate.

3. Recoveries of the order of ± 0.05 mg. magnesium/100 ml. are obtained over the normal ranges of plasma or serum magnesium concentration.

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The Initial Stages in the Purification of Pig Liver Esterase

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The early work of Simonds (1919) demonstrated that there was considerable difficulty in obtaining a clear solution of liver esterase, because this enzyme was very firmly bound to the insoluble cell substances. Even autolysis in glycerol solutions for two to three years did not completely liberate it.

Willstätter & Memmen (1924) improved the yield somewhat by introducing the acetone dried powder preparation from which the esterase could be extracted with dilute NH_4OH . Owing to the losses involved in the acetone dried powder procedure and to its inconvenience, Bamann, Mukherjee & Vogel (1934) reinvestigated the extraction from the fresh liver and obtained a greater yield of enzyme more conveniently. They added dilute NH_4OH to the minced tissue and allowed it to autolyse at room temperature for 3–4 days, after which the mixture was made weakly acid with dilute acetic acid and

the supernatant filtered off. Complete extraction of the enzyme was claimed, and the solution was 2–3 times purer than the original fresh liver.

In this paper some properties of the bound esterase are described, and a method is given of increasing the purity of the enzyme by making use of its insoluble state in the fresh liver. By diluting the liver mince with water, collecting the insoluble material in a high-speed centrifuge and washing it twice more in a similar manner with water, it was possible to reduce the concentration of soluble impurities to less than 1% of their initial concentration. Solubility tests of the type used by Butler & Montgomery (1933) and by Roche, Dorier & Samuel (1936) were also carried out both on the esterase content of the extract prepared by the method described in this paper and on that of Bamann *et al.* mentioned above.