Determination of Magnesium in Tissue by the Titan Yellow Method in the Presence of Silver and Mercury.* (27477)

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The procedures described here were developed in connection with studies on the effects of x-irradiation on the chemical composition of brain tissue(1). Silver and mercury ions are usually present in such small amounts in biological materials that they do not interfere with Mg determination by the Titan yellow method. If they are added during the processing of material for subsequent analysis, however, interference with Mg determination will occur. The elimination of Ag or Hg by a simple precipitation process such as silver chloride or mercury sulfide is not sufficient, and traces of these metals remaining in solution are enough to interfere with Mg determination. The present work was undertaken in an effort to eliminate these difficulties. Since this paper does not deal primarily with determination of Mg, other reports on the Titan yellow method(*e.g.* 2) should be consulted.

Interference by silver and mercury. To 2.0 ml of a solution containing a known amount (0.2-1.2 μeq.) of Mg, the following were added with continuous stirring: 1.0 ml of 1% polyvinyl alcohol (Du Pont's Evanol), 0.5 ml of 14 mg % Titan (Clayton) yellow solution and 1.0 ml of 15% NaOH solution. After approximately 12 hours, the optical density was read in a recording spectrophotometer (Spectracord, Warren Electronics Inc.)‡ against a Mg-free blank solution. In the absence of Ag and Hg, the absorption maximum of the solutions containing varying amounts of Mg is always found at 550 mμ and all spectral curves intersect at 500 mμ (Fig. 1A). A linear relationship exists between the 550 mμ reading and the Mg concentration except when the latter is in excess to saturate the dye present (Fig. 1B).

If Ag or Hg is added to the solutions, the reading at 550 mμ becomes depressed (Fig. 2A) even though an excess amount of Mg is present. As the amount of Ag or Hg increases, the intersection of the curves at 500 mμ is also abolished (Fig. 1A), hence a check reading at this wave length may be used to detect the presence of either of these ions. If Mg is absent and only Ag or Hg reacts with the dye solution, the optical density of the solution read against a water blank decreases as the amount of Ag or Hg is increased (Fig. 2B). As seen in Fig. 2C, a fairly linear relationship is obtained when the diminishing optical densities are plotted against the corresponding increasing concentrations of Ag or Hg. Since extremely small amounts of Ag (*e.g.* 0.006 μeq/ml) or Hg can cause a considerable decrease in the optical density and since this effect seems to be specific for these 2 metals, this reaction may possibly be used for quantitative determination of traces of these substances.

Attempts to eliminate the interference of Ag or Hg by making spectrophotometric readings at some wavelength other than 550 mμ failed because the errors proved to be too large. To prevent free Ag ions from reacting with the dye, the addition of sodium thiocyanate, many times the stoichiometric amount, proved effective.

Elimination of Ag interference with thiocyanate. In the train of analytical procedures used in this laboratory for brain tissue(1), excess Ag is added for Cl determination and it must be removed by the following procedure before Mg determination. The Ag is first largely precipitated as chloride. To the resulting solution, which contains only traces of Ag, enough sodium thiocyanate solution is added so that it represents 5-7% of the final volume. Identical amounts of sodium thiocyanate solution are added to the standard

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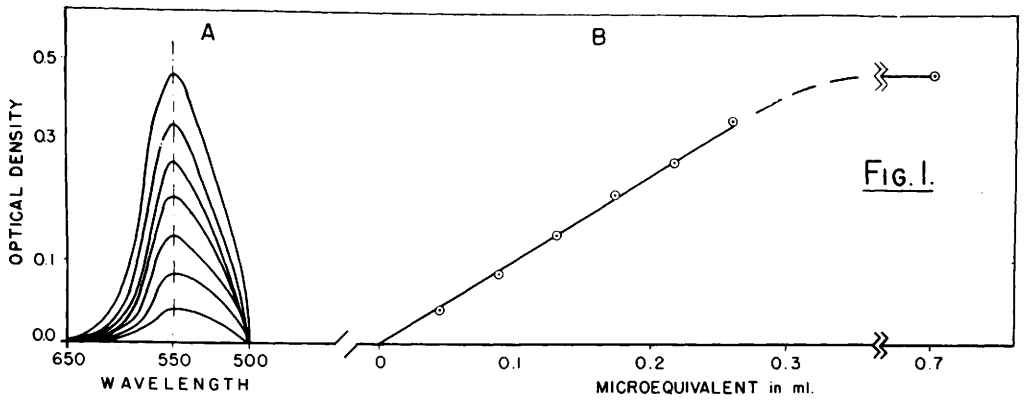

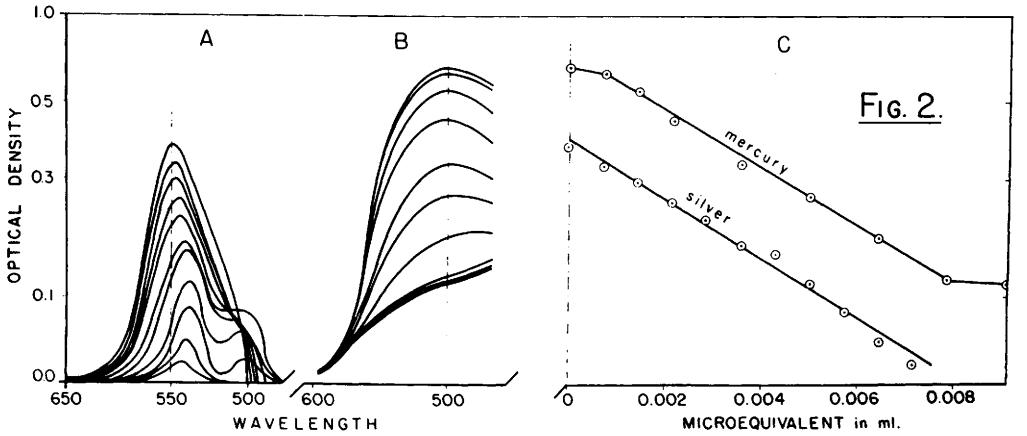

FIG. 1. (A) Absorption curves of solutions containing different concentrations of magnesium in Titan yellow dye solutions of constant concentration read against a Titan yellow blank. (B) Showing that a linear relationship exists between concentrations of magnesium and optical density readings until "saturation" is reached.

FIG. 2. (A) Optical densities of magnesium "saturated" dye solutions containing different amounts of silver read against a magnesium-free and silver-free dye solution as blank. The dye and magnesium concentrations are constant but optical density at 550 $m\mu$ diminishes with increased silver concentrations. (B) Optical densities of magnesium-free dye solutions containing different amounts of mercury read against water. The action of mercury is similar to silver. Optical densities of solutions with equal dye concentrations diminish until the dye is "saturated" and no further decrease is recorded. (C) Optical densities of the peaks of curves shown in Fig. A and B are here plotted as functions of the concentration of mercury and silver expressed in microequivalents/ml.

and blank solutions. That this procedure is effective in removing the Ag interference was demonstrated in the following experiments. A known amount (0.5-2.0 g) of brain tissue was digested with 15 ml of approximately 20% nitric acid and the solution after digestion was made up to 50 ml. To a 25-ml aliquot of this digestion solution, 5 ml of 0.02 N $AgNO_3$ solution and 5 ml of 4% HCl were added, and the resulting AgCl precipitate was separated by filtration. Nine 3-ml samples of the filtrate were taken. To another 25-ml aliquot of the digestion solution, 10 ml of water was added and three 3-ml samples were taken. After the solutions were dried, the organic matter was destroyed by repeated additions of a few drops of concentrated nitric acid during heating and the dry residue treated as follows: To the 3 Ag-free samples, as well as to 3 of the 9 Ag-added samples, 2.0 ml of 12% sodium thiocyanate solution was added. Results on Mg determination of these samples (Table I) clearly show that traces of Ag remaining after HCl precipitation prevent the detection of any Mg (Group II) and that addition of sodium thiocyanate (Group III)

TABLE I. Use of Sodium Thiocyanate in Eliminating the Interference by Silver during Magnesium Determination.

Group	No. of samples	Addition of: $AgNO_3$ and Ag precipitation with HCl	Addition of: NaSCN	Mg in brain tissue (meq/kg fresh wt): Mean	Mg in brain tissue (meq/kg fresh wt): Range
I	3	—	—	10.3	(9.9-10.8)
II	3	+	—	.0	(.0- .0)
III	6	+	+	10.0	(9.2-10.2)

eliminates this interference such that the results on Mg agree well with the control samples (Group I). Furthermore, the thiocyanate-treated-Ag-containing samples showed the characteristic spectral curves with intersection at 500 mμ, as those shown in Fig. 1A. This was not the case in the samples containing traces of Ag but not treated with thiocyanate.

Summary. The interference of Ag and Hg in determination of Mg by the Titan yellow dye method is demonstrated. A method is described for prevention of Ag interference by use of sodium thiocyanate and illustrated with analysis on brain tissue.

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Plasma Free Fatty Acids During Exercise and the Effect of Lactic Acid. (27478)

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Investigations of recent years seem to focus attention on the possible role of unesterified free fatty acids (FFA) as an extremely rapidly moving fuel(1,2). Andres and coworkers(3) have shown that O_2 uptake of the skeletal muscle is so high that it cannot be explained by oxidation of glucose alone. Direct electrical stimulation induces a measurable uptake of FFA by skeletal muscle of the dog(4). Friedberg *et al.* reported(5) a decrease of plasma FFA and an increased disappearance rate of injected palmitic-1-C^{14} during exercise.

To obtain further information about the role of FFA in muscular work, we studied the effect of exercise on plasma FFA levels on dogs with indwelling arterial and venous catheters.

Methods. Experiments were carried out on healthy mongrel dogs 9-13 kg of body weight. In morphine-pentothal narcosis, polyethylene catheters were placed into the right carotid artery and jugular vein. After 2-3 days of recovery, the dog was put on the treadmill, working for 20-25 minutes at a speed of 100 m/min and at a grade of 15-19%. O_2 uptake and CO_2 output were measured as follows: A plastic hood with a rubber collar was placed over the head of the animal. An air flow at rest of 25 l/min and 120 l/min at work was maintained by a pump; samples of the diluted expired air were collected in Douglas bags for about 2 minutes. To assure a proper evaporation of water from the tongue of the dog, the air flow was increased 5-6-fold between sample collections. The diluted expired air was analyzed by means of a Noyons diaferometer, the calibration of which was carried out as previously described(6). Arterial blood samples were taken at rest, during exercise (usually at 1½ min, 10 minutes and during the last 2 minutes of work), as well as 10 and 40 minutes after work. In other experiments 10 or 20% lactic acid was infused in the resting dog into the jugular vein at a rate of 1.97