slices are used. Despite the fact that approximately equal weights of adrenal tissue from the same adrenal and equal quantities of radioactive corticosterone were present, Experiment B had twice the specific activity of Exp. A, but similar microgram amounts of aldosterone. It is difficult to interpret this quantitative discrepancy.

Cell permeability, precursor pool size, and state of enzyme activity are important variables in *in vitro* steroid synthesis. For ideal comparisons of different precursors, the same adrenal tissue should be studied simultaneously, using equimolar tracer quantities of precursors with identical specific activities; and rates of synthesis of aldosterone with its immediate precursors determined.

Conclusions. Synthesis of aldosterone by human adrenal slices from corticosterone-4-C^{14} has been demonstrated *in vitro*.

1. Kahnt, F. W., Neher, R., Wettstein, A., *Experientia,* 1955, v11, 446.

2. Rosemberg, E., Rosenfeld, G., Ungar, R., Dorfman, R., *Endocrinology,* 1956, v58, 708.

3. Ayres, P. J., Hechter, O., Saba, N., Simpson, S., Tait, J. F., *Biochem. J.,* 1957, v65, 229.

4. Travis, R. H., Farrell, G. L., *Endocrinology,* 1958, v63, 882.

5. Seltzer, H. S., Clark, D. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 674.

6. Burton, R. B., Zaffaroni, A., Keutmann, E. H., *J. Biol. Chem.,* 1951, v188, 763.

7. Bush, I. E., *Biochem. J.,* 1952, v50, 370.

8. Abelson, D., Bondy, P. K., *Arch. Biochem.,* 1955, v57, 208.

9. Eberlein, W. R., Bongiovanni, A. M., *ibid.,* 1955, v59, 90.

10. Kliman, B., Peterson, R. E., *Fed. Proc.,* 1958, v17, 255.

Received May 4, 1959. P.S.E.B.M., 1959, v101.

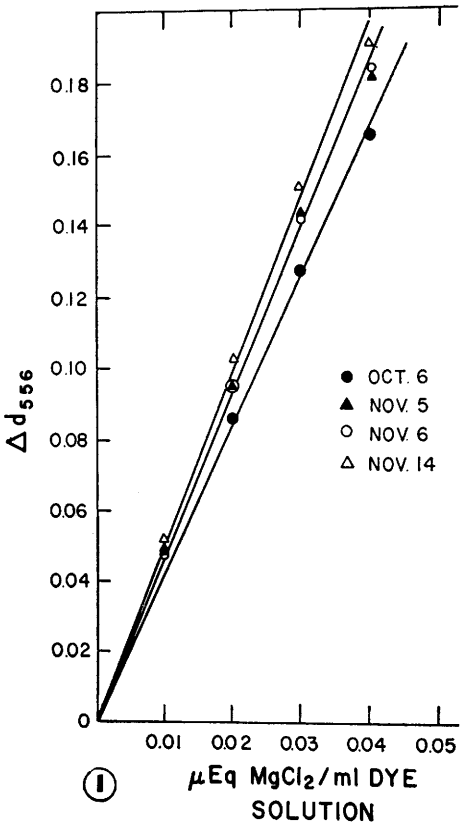
Analysis of Serum Magnesium in Presence of Calcium with Chrome Fast Blue BG. (25077)

WILLIAM J. BOWEN AND HAROLD L. MARTIN (Introduced by H. Specht)
Nat. Inst. of Arthritis and Metabolic Diseases, N. I. H., U. S. Dept. of H. E. W., Bethesda, Md.

Colorimetric methods for determination of magnesium have utilized principally a series of yellow dyes(1) (Titan yellow, brilliant yellow, thiazol yellow, Clayton yellow) and Eriochrome Black T(2). Of the yellow dyes, Titan yellow has been widely accepted, but, as with all yellow dyes, a stabilizer such as gum ghatti, or polyvinyl alcohol must be used and pH values of 12.8 or more maintained. Methods using other dyes require removal of calcium and/or elapses of time for color development. Use of azo dye, 1, 8-dihydroxy-2-(3'-chloro-6'-hydroxybenzene azo) naphthalene-3-6-disulfonic acid, or Chrome Fast Blue BG, requires no removal of calcium, no waiting for color development, and no stabilizers. It is sensitive to quantities as small as 0.01 microequivalents of magnesium/ml of dye solution. This dye was first used by Japanese workers and was introduced in this country by Carson (3). Its application to analysis of magnesium was initiated in this laboratory by Lewis and Kerwin(4) and has now been refined.

Methods. Chrome Fast Blue BG was obtained from American Cyanamid Co., Bound Brook, N. J., and used after further purification.* An aqueous solution of 0.001 M (molecular weight of Na salt = 518.5) was prepared and can be stored several months. To 7 ml of 0.001 M Chrome Fast Blue BG was added 10 ml of 1 M ethylamine buffer (adjusted to pH 11.1 with HCl) and 83 ml of water. To 3 ml of this buffered dye solution in a Corex cuvette was added solutions of 0.2% trichloroacetic acid (TCA) for blanks, of $MgCl_2$ in 0.2% TCA for standardization (Fig. 1), and of the deproteinized serum (see below) for determination of Mg-content of serum. All optical densities were obtained with Beckman DU spectrophotometer. A standard

* Procedure for purification is available from Am. Cyanamid Co.



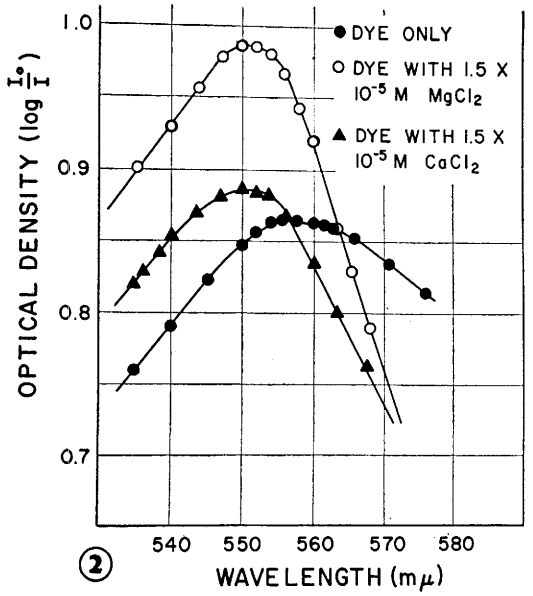


FIG. 1. Standard curves of 4 different days. $MgCl_2$ in 0.2% trichloroacetic acid.

FIG. 2. Plots of absorption spectra of Chrome Fast Blue BG with and without calcium and magnesium.

curve was made each day with buffered dye prepared for that day. Blood serum was deproteinized by either of 2 methods, the choice depending upon volume available.

Method 1. When several ml of serum were available, it was made 4% TCA by adding one volume of 20% TCA to 4 of serum. It was mixed by stirring with glass rod. The mixture was heated in water bath at 90-95°C for 5 min. and then centrifuged. To one volume of supernatant fluid was added 19 volumes of demineralized water thus making the concentration of TCA 0.2% and Mg and Ca content 1/25 of that in original serum. *Method 2.* Small volumes of serum (ca 0.1 ml) were made 4% TCA by adding equal volume of 8% TCA. This mixture was stirred to break up coagulated protein and the tube set in water at 90-95°C for 5 min after which the volume was increased to 40 times the original volume of serum with demineralized water, thus making the concentration of TCA 0.2%. The suspension was filtered through as small a circle of ashless paper as practical. Whichever method was used to deproteinize, every effort was made to remove all protein because any remaining in the supernatant solution, or filtrate, would go back into solution and bind magnesium when it was put into the alkaline dye solution. The diluted supernatant fluids were mixed for analysis by procedure given above. The difference in optical density (Δ d) between this mixture and a blank solution composed of 3 ml of buffered dye and 1 ml of 0.2% TCA was obtained at a wave length between 555 and 557 mμ (see below). Amount of magnesium in serum was ascertained from standard curve (Fig. 1) of Δ d and by calculation with proper dilution factor. This method for analyzing blood serum for magnesium without removing calcium depended upon obtaining the difference in optical density between dye with and without deproteinized serum at a wave length isosbestic (Fig. 2) for dye plus calcium and dye alone. This wave length was usually within 0.5 mμ of 556.5 mμ and was ascertained precisely for each preparation of dye by exploring between 555 and 557 mμ for that wave length which gave a Δ d value of zero, between a cuvette filled with 3 ml of dye and 1 ml of 0.2% TCA

TABLE I. Magnesium in Blood Sera of 4 Groups.

No. of determinations	Range of values (meq/l)	Mean (meq/l)
8	1.41-2.05	1.80
5	1.72-2.04	1.90
12	1.15-2.57	1.69
12	1.41-1.76	1.55

and one filled with dye plus 10^{-7} moles of $CaCl_2$ added in 1 ml of TCA. The method was tested by ascertaining amounts of magnesium that could be recovered from whole and deproteinized sera to which $MgCl_2$ was added. Preliminary tests showed that when varying amounts of $MgCl_2$ were added to whole serum, about 100% could be recovered. Two extended tests were then done in which added amounts of $MgCl_2$ were varied over the range of Fig. 1. Magnesium was added to deproteinized sera. Slopes of least square lines indicated that recovery rate in one test was 93.5 $\pm$ 4.5% and in the other 98.7 $\pm$ 3.1%.

Results. Values obtained by this method for amount of magnesium contained in blood sera are given in Table I. These sera were obtained from patients in the Clinical Center, National Institutes of Health.

Table I gives the range of individual values of magnesium in meq/l and the means of magnesium-contents of 37 sera done in 4 groups of determinations. Some ranges are large, *e.g.*, group No. 3. The lowest value of 1.15 meq/l, however, was obtained in 2 other analyses. The same applies to highest value 2.57, *i.e.*, high values were also obtained in 2 other analyses.

Mean values of magnesium in these blood sera compare well with those already in the literature. Some of these in meq/l are 1.66 (5), 1.89(6), 1.75(7), 1.58(8), and 1.80(9).

An advantage of this method is that the calcium of the specimen does not have to be removed. To make sure that calcium was not interfering, $CaCl_2$ was added to solutions used for standardization so that the Ca : Mg ratio ranged from 10:1 to 0.1:1. Such additions of calcium did not change the Δ d_{556} of buffered dye solutions containing 0.01, 0.015 and 0.02 meq of magnesium/l. Furthermore, all specimens of serum lots 3 and 4 of Table I were done with and without decalcification. Calcium was removed by making undiluted serum 0.8% ammonium oxalate. Portions of sera from which calcium was removed had mean magnesium-contents of 1.81 and 1.63 meq/l, while whole sera had 1.69 and 1.55 meq/l.

Summary. 1. A method is described for determination of magnesium in blood serum using the azo dye Chrome Fast Blue BG. 2. Use of this dye affords immediate determination of serum magnesium without removal of calcium and with no addition of stabilizers. 3. The dye as used here is sensitive to as little as .01 microequivalent of magnesium. 4. Thirty-seven sera were analyzed. These had an over-all magnesium-content of 1.73 meq/l of sera.

1. Ericsson, Y., *J. Dental Research,* 1955, v34, 104.
2. Levine, R. M., Cummings, J. R., *J. Biol. Chem.,* 1956, v221, 735.
3. Carson, Paul, *Fed. Proc.,* 1953, v12, 187.
4. Lewis, M. S., Kerwin, T. D., *ibid.,* 1954, v13, 89.
5. Hunter, G., *Nature,* 1958, v182, 263.
6. Orange, M., Rhein, H. C., *J. Biol. Chem.,* 1951, v189, 379.
7. Smith, A. J., *Biochem. J.,* 1955, v60, 522.
8. Smith, R. G., Craig, P., Bird, E. J., Boyle, A. J., Iseri, L. T., Jacobson, S. D., Myers, G. B., *Am. J. Clin. Path.,* 1950, v20, 263.
9. Standard values in blood. Edited by E. C. Albritton, W. B. Saunders Co., Phila., 1952, p117.

Received May 5, 1959. P.S.E.B.M., 1959, v101.