are not shown in Table I. Similarly, the data on the capillary coagulation time are not included in the table, since the results paralleled those of the tube coagulation time. The hematocrit estimations showed no characteristic variations and the results, accordingly, are not given in the table.

In 2 normal dogs used as controls, 5 prothrombin estimations over a 2½-hour period showed no greater variation than 0.5 seconds in the prothrombin time. In addition, 3 other control animals were used; 2 animals were injected with sodium chloride and 1 animal with potassium chloride. None of these animals developed the characteristic fall in the prothrombin and coagulation times, that were invariably obtained when sodium citrate was injected. The hematocrit determinations in the normal controls, as well as in the animals injected with sodium or potassium chloride, showed no characteristic variations.

Summary. Intramuscular injections of sodium citrate caused a lowering of the clotting time of blood as determined by the capillary and Lee and White methods. The increased coagulability was accompanied by a fall in the prothrombin time in the blood, as determined by Quick's method. Control animals, and those injected with sodium or potassium chloride, did not show a lowering of the prothrombin time.

11914

An Error in Measuring Changes in Plasma Volume After Exercise.

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The plasma volume under basal conditions may be satisfactorily determined by the dye method. The original dye method described by Keith, Rowntree, and Geraghty,[1] has been rendered more accurate by the use of better methods of colorimetry and by the calculation of the volume from the disappearance slope of the dye. It is impossible to measure rapid changes in plasma volume which last only a short

[1] Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, **16**, 547.

time by repeated injections of the dye. This is true because changes in plasma volume occurring during the determination dilute or concentrate the residual dye present in the serum from the previous injection. To overcome this difficulty, a method has been developed for measuring rapid fluctuations in plasma volume using a single injection of dye.[2, 3] The disappearance slope of the dye under basal conditions is first determined and changes in optical density above or below this slope, which are induced by experimental procedures, are taken as indicating a decrease or increase in plasma volume. The change in plasma volume is calculated from the ratio of the optical density of the dyed serum to the optical density of the corresponding point on the disappearance slope.

This method has been used to measure the changes occurring in plasma volume after exercise.[4, 5] In studying the effect of exhausting exercise on the plasma volume of human subjects, we noted that the decrease in plasma volume, as measured by this method, did not agree with that obtained by calculating the change in plasma volume from the increase in serum protein concentration. In attempting to find a cause for this discrepancy it was thought possible that the optical properties of the dye-free serum might change during exercise, thus leading to an error. In order to determine whether this occurred, subjects were exercised on a bicycle ergometer to the point of exhaustion. The usual period of exercise was 5 minutes. Samples of blood were taken without stasis before and after exercise. The serum obtained before exercise was compared with the serum obtained after exercise in the Evelyn photoelectric microcolorimeter, using a filter with maximum light transmission at 620 millimicrons.[6, 7] Both samples of serum were also compared with distilled water. It was found that the serum obtained after exercise had a lower galvanometer reading than the serum obtained before exercise. The increase in L value (2—log. galvanometer reading) of the serum after exercise is shown in Table I. A similar increase in Δ D value of serum after exercise was found with the Koenig-Martens spectrophotometer using a light source with a wave length of 620 millimicrons.

The exercise experiments were repeated in 5 cases after the injec-

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TABLE I.
Effect of Exercise on L Value* of Serum as Measured by the Photoelectric Microcolorimeter.

Subject	Increase in serum protein after exercise (g per 100 cc)	Increase in L value* of serum after exercise
J.G.	1.3	.017
R.E.	1.3	.034
H.W.	.5	.021
M.M.	.7	.005
L.H.	1.2	.013
R.E.	.8	.019
Avg	0.96	.018

*L value = 2 — log. of galvanometer reading.

tion of dye, in order to decide whether the change in the optical properties of the dye-free serum caused a significant error. Using 10 mg of Evans blue, the average increase in L value of the serum after exercise was .045. The average change in L value of the serum of the subjects who had not received dye was .018. Thus, 40% of the change in L value usually attributed to an increase in dye concentration was actually due to a change in the L value of the serum. The decrease in plasma volume which occurs after exercise is exaggerated by the dye method. This error has not been taken into account by recent workers using the dye method for determining changes in plasma volume after exercise.[4, 5] If this method is to be used for determination of changes in plasma volume produced by other procedures than exercise, it must first be demonstrated that the optical properties of the dye-free serum are not altered by the procedures.

The error caused by the change in optical properties of the serum varies inversely with the amount of dye used. If 20 mg of dye had been injected instead of 10 mg, the error from the change in optical properties of the serum would have been reduced by half. It is not practical to use more than 20 mg of Evans blue in human subjects, because larger quantities of the dye may cause discoloration of the skin. In animal experiments, however, this error can be minimized by using larger amounts of dye.

Summary. When the dye method is used for measuring changes in plasma volume after exercise, an error occurs due to changes in the optical properties of the serum.