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Determination of Diodrast in Whole Blood.*

MILTON E. BOBEY AND J. WAIDE PRICE. (Introduced by J. T. Wearn.)

From the Department of Medicine, Western Reserve University, and Lakeside Hospital, Cleveland, Ohio.

In most kidney function studies involving the use of diodrast (D), this substance is determined in terms of its iodine content (I) in plasma and in urine. In some cases, however, analysis of D in whole blood is desirable and, aside from tedious ashing procedures, the only method proposed for the determination of blood D is that of White and Rolf[1] which requires a large correction factor (about 50% of the I determined) and which should be evaluated for each experiment.

The method described by Alpert[2] gives complete recovery from 1:15 zinc hydroxide filtrates of plasma containing 1-4 mg of I per 100 cc but was not applied to whole blood analyses. Direct use of this plasma method with whole blood yields a filtrate containing on the average only about 85% of the D present in the blood. The recovery may be increased but not to 100% by the use of greater dilutions, up to 1:100, in the preparation of whole blood filtrates, but this either reduces the accuracy of the analysis by lowering the amount of I in each determination or else necessitates a modification of the procedure to the determination of smaller amounts of I which largely nullifies the chief advantage of the method, its simplicity. It has been observed, however, that the recovery is inversely proportional to the cell volume as determined by hematocrit and the use of a simple recovery factor based on this observation gives the true whole blood I value within the range of accuracy of the method as applied to plasma.

The correction factor necessary to the use of Alpert's method with whole blood was obtained as follows:

Procedure. Portions of oxalated dog blood were mixed with plasma or cells from the same animal to vary the cell volume. To these bloods D, dissolved either in normal saline solution or in plasma was added so that the final cell volume ranged from 15.8 to 48.5% and the I level was equivalent to 2-20 mg %. The samples then stood for one hour at 38°C with occasional shaking to allow diffusion of D into the cells. White[3] has shown that this diffusion takes place slowly (presumably at room temperature). At 38°, however, 40 min suffices for attainment of a diffusion equilibrium between cells and plasma of the order observed in arterial blood *in vivo*. The preparation of filtrates from these bloods, digestion and titration were carried out exactly as described by Alpert. Hematocrit determinations were also done. From the known amount of D present in each sample, the percentage recovery could be calculated and correlated with the cell volume. The results of 24 determinations in 5 experiments are shown graphically in Fig. 1. The straight line fitted to the points by the method of least squares is given by the equation:

$$\% \text{ Recovery} = 99.8 - 40.0 \text{ Vc}$$

where Vc is the cell fraction as determined by hematocrit. The line extrapolates to 99.8% recovery when Vc = O (plasma) and to 59.8% recovery when Vc = 1.00 (cells). The validity of the latter figure has not been tested since the precipitating reagents do not completely remove the cell protein when employed in the proportions used for whole blood and plasma. The 99.8% recovery indicated for plasma has been verified experimentally and is in agreement with the re-

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1 White, H. L., and Rolf, D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 1.

2 Alpert, L. K., *Bull. Johns Hopkins Hosp.*, 1941, **68**, 522.

3 White, H. L., *Am. J. Physiol.*, 1940, **130**, 654.

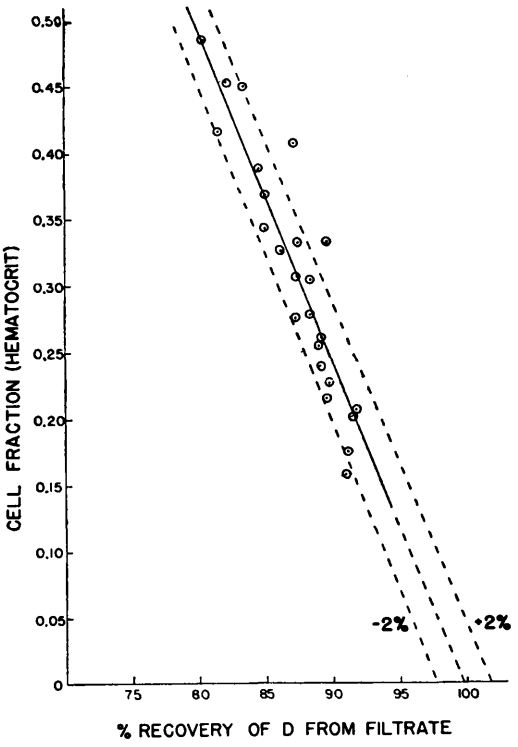

FIG. 1.

The relation between the per cent recovery of D from 1:15 whole blood filtrates and the cell fraction as determined by hematocrit. The broken lines enclose the area representing a deviation of ±2% from the theoretical value given by the equation discussed in the text.

coveries reported by Alpert for plasma levels of 1-4 mg per 100 cc. When higher levels were used in this work, up to 20 mg per 100 cc, the filtrates were diluted to be equivalent to the lower range and the recoveries were equally good. Of the 24 determinations, one deviates from the average line by +4.4%, one by +3.7%, one by —2.7%, and the remaining 21 are within ±2%.

The true whole blood I value then becomes:

$$\frac{\text{I determined in whole blood filtrate}}{99.8 - 40.0\ \text{Vc}} \times 100$$

Discussion. The correction factor discussed here applies only to dog blood. In man, the D content of cells in equilibrium with plasma is only about half of the D content of dog cells in similar equilibrium and the rate of diffusion from plasma to cells is lower.[4] Whether or not these differences would significantly change the correction factor should be determined before applying the correction to human blood or to blood of any other species. In the case of the dog, however, the recovery of D from the laked blood is dependent only upon the cell volume, regardless of whether the D was present originally in plasma alone, cells alone or was in equilibrium between the two. This presumably indicates that the recovery of D in the filtrate is dependent on the total protein present in the laked blood solution at the time of precipitation rather than on the location of the D at the time of laking, the recovery being essentially complete in the presence of plasma protein but decreasing with increase in the amount of cell protein. This influence of protein may be involved in the incomplete recoveries obtained from plasma, whole blood and cells in the method of White and Rolf.

Conclusion. Alpert's method for the determination of D in plasma may be applied to the determination of D in whole blood of dogs by the use of a simple correction factor based on the hematocrit determination of cell volume.

[4] White, H. L., Findley, T., Jr., and Edwards, J. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 11.