

## 13148

**Confirmation *in vitro* of Griffith-Campbell Method for Measuring Blood Volume.**

J. Q. GRIFFITH, JR.\*

*From the Robinette Foundation and the Medical Clinic of the Hospital, University of Pennsylvania.*

Beckwith and Chanutin<sup>1</sup> state that "in the hypertensive partially nephrectomized rat, the plasma volume was increased, the red cell volume decreased and the total blood volume remained unchanged." In referring to a paper by Griffith and Ingle,<sup>2</sup> who found an elevated blood volume in hypertensive partially nephrectomized rats, they say, "On inspection of their procedure for estimating blood volume, it appears that they neglected to take the red cell volume into consideration and the values reported by them probably represent plasma volumes . . . the increased plasma volumes (found by Beckwith and Chanutin) . . . agree with the reinterpreted data of Griffith and Ingle."

The blood volume method used by Griffith and Ingle was that previously reported by Griffith and Campbell.<sup>3</sup> In this method 0.3 cc of 5% vital red is injected intravenously and 4½ minutes later 50 cmm of whole blood are removed and placed in 2.95 cc of physiologic saline, the resulting volume being 3 cc and the blood dilution 1:60. After centrifuging, 2 cc of the supernatant fluid are removed and compared in a colorimeter with suitable standards. The red cells remain in the 1.0 cc left in the test tube, and a slight error is thus introduced in that the volume which the cells occupy is included in the 3 cc but does not contain an aliquot portion of the dye. The dye is actually contained in 3 cc less the volume of the cells. If the cells comprised 50% of the total blood, the figure would be 3.0 cc less .025 cc or 2.975 cc. This represents an error of 0.8%, and would make the calculated figure too low by that amount. However, the accuracy of the method is probably only about 5%, and such a correction appears unnecessary. Moreover, the range of variable concentrations of red cells consistent with life is such that the highest

---

\*Atwater Kent Fellow in Medicine.

<sup>1</sup> Beckwith, J. R., and Chanutin, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **46**, 66.

<sup>2</sup> Griffith, J. Q., and Ingle, D. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **44**, 538.

<sup>3</sup> Griffith, J. Q., and Campbell, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 38.

red cell volume would differ from the lowest by somewhat less than 25 cmm. In comparisons of blood volumes for different animals the actual error from omitting this correction would probably be less than 0.5%.

To confirm this *in vitro*, the following experiment was performed: (1) To 5 cc of citrated blood having an hematocrit value of 43, 0.3 cc of 5% vital red was added and, after thorough mixing, 50 cmm of the citrated blood with the dye were removed and carried through the procedure detailed above. The value was found to be 4.9 cc. (2) Five cc of citrated blood mixed with serum to give an hematocrit value of 18 was carried through the same procedure. The value found was 4.7 cc. (3) Five cc of serum were treated similarly. The value found was 5.0 cc.

Thus the differences between the results of Beckwith and Chanutin and those obtained by Griffith and Ingle cannot be explained by the latter's misconception of their blood volume method. Certain further points should also be kept in mind: (1) Chronicity of the experiment. Beckwith and Chanutin used animals operated 3 to 4 months previously. Blood pressures were not measured but hypertension was "judged by the tortuosity and dilatation of the carotids." Griffith and Ingle used animals approximately 2 weeks after the second operation or 3 weeks after the first. They actually measured blood pressure, and not all animals were hypertensive. Tortuosity and dilatation of the carotids were not noted. (2) The presence or absence of anemia. Beckwith and Chanutin's rats were anemic. Griffith and Ingle did not measure the red cell volume in the animals whose blood volume was measured, but similar animals did not show a significant degree of anemia as compared with controls. If a normal red cell volume were added to the plasma volume figures of Beckwith and Chanutin, the results for blood volume would approach those of Griffith and Ingle. (3) Technical differences. The two groups of workers used different dyes and different times for collecting samples. Griffith and Ingle removed less renal tissue in the second operation than did Beckwith and Chanutin and many of their animals recovered from their hypertension, whereas those of the latter apparently did not.