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A Semi-Permanent Acid Hematin Standard for Hemoglobin Determinations with Colorimeter.

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The standard was prepared as follows: 15.5 mg. of a commercial grade of hemin were dissolved in 50 cc. of 0.1 *N* NaOH. To this mixture was added 25 cc. of a 5% solution of gelatin (to prevent precipitation of the hemin from acid solution),¹ in a liter volumetric flask and made up to the mark with 0.1 *N* HCl. The resulting solution of acid hematin was compared with the Bausch & Lomb brown filter for hemoglobin determination in a Bausch & Lomb colorimeter. The reading was found to be 14.5 mm. Since it was desired that the reading should be 20 mm., 14.5 cc. of the standard were made up to 20 cc. with 0.1 *N* HCl. The resulting solution, placed in the right cup of the colorimeter and compared with the standard slide, gave a reading of 20 mm. A colorimeter cup with fused-on top and bottom 20 mm. apart and side neck for filling² had the side arm drawn out and constricted until an opening of about 2 mm. remained. The standard hematin solution was introduced into this opening by means of a piece of glass tubing drawn out to a capillary point until the cup was completely filled and the side arm about three-fourths full of liquid. The constricted end of the side arm was dried with a piece of filter paper and sealed off. The standard thus prepared matched exactly the standard Bausch & Lomb glass slide. It is believed that the solution prepared as described above will remain standard for about a year, at the end of which time the side arm may be drawn out and reopened, and a new standard introduced into the colorimeter cup.

On the basis of molecular weight calculations it was observed that the commercial hemin sample used was approximately 43% hemin.

¹ Elvehjem, C. A., *J. Biol. Chem.*, 1931, **93**, 203.

² McClendon, J. F., Russell, Sidney, and Tracy, Edward, *J. Biol. Chem.*, 1926, **70**, 705.