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## Protein Determination for Large Numbers of Samples

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LOWRY, Rosebrough, Farr, and Randall's procedure for colorimetric determination of protein [*J. Biol. Chem.* **193**, 265 (1951)] is particularly convenient when only a few samples are determined at one time.

Two modifications in procedure make it possible to determine large numbers of samples. Use of a comparatively smaller volume of more concentrated alkaline copper reagent and a larger volume of more dilute Folin phenol reagent permits introduction of a proportionately larger volume of phenol reagent with sufficient force to ensure adequate preliminary mixing, and final mixing can be postponed until after the reagent has been added to all the samples. Heating the final mixtures of

samples and reagents at 50° C. accelerates development, reducing the time to minutes.

One-milliliter aliquots of alkaline copper reagent composed of 10 parts of 10% sodium carbonate in 0.5*N* sodium hydroxide and 1 part of 0.5% copper sulfate in 1% potassium tartrate are added to 1-ml. aliquots of protein solution in colorimeter tubes 14 mm. in outside diameter. After the mixtures have stood for 10 minutes, 3-ml. aliquots of a 1 to 11 dilution of Folin phenol reagent are added to the samples as forcibly as practicable. The mixtures of samples and reagents are heated for 10 minutes at 50° C. in a constant temperature water bath. After the mixtures are cooled to room temperature, absorbance is read at wave lengths of 540 to 750

m $\mu$ , depending on the sensitivity required.

For addition of the alkaline copper reagent and the Folin phenol reagent, hand-operated plunger-type pipets or motor-driven automatic pipets are very useful. With automatic pipets, glass instead of stainless steel valves are required when the phenol reagent is used, because of the problem of corrosion.

Entirely analogous results were obtained with bovine serum albumin and with gelatin, although, as expected, the color with gelatin was about half that with bovine serum albumin. The reproducibility of analyses over the range of 0.04 to 0.20 mg. of protein in the modified test is, on the average, to 2%, essentially the same as in the original test.