The phytin that we obtained corresponded to the requirements of the State Pharmacopoeia [3] and contained more than 45% of P_2O_5.

The phytin was determined in the following way. A 25-g sample of roasted rice bran was covered with 75 ml of 1% nitric acid containing 20% of ethanol, and the mixture was stirred for 30 min. The suspension was filtered through a Schott funnel, pressed out, and washed with 25 ml of the same acid. The meal was treated twice more with 50 ml of acid each time (30 min), 25 ml of the acid being used for washing. The combined extracts were filtered through filter mass (∿2 g) and the resulting clear yellow solution was carefully made alkaline with 25% ammonia. The phytin in the precipitate was filtered off by filtration through a Büchner funnel with a double filter paper. The filtered-off phytin paste was dissolved in 8-10 ml of 25% HNO_3 and the acid solution was filtered through a paper filter and [the phytin was reprecipitated with ammonia and filtered off, and the precipitate was] washed with water and thanol and was then dried. The yield of phytin was 1 g, which amounts to 4% of the weight of the rice flour. The dried phytin consisted of a white powder insoluble in water but soluble in dilute mineral acids and also in 1% nitric acid containing 20% of ethanol.

LITERATURE CITED

1. A. M. Sobolev, Usp. Biol. Khim., 4, 248 (1962).
2. S. Babakhodzhaeva, N. Rizaev, and A. Gorokhovskaya, Khim. Farm. Zh., 2, No. 4, 42 (1968).
3. State Pharmacopoeia of the USSR [in Russian], Xth edition, Moscow (1968), p. 539.

A SIMPLE DETERMINATION OF PROTEIN

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In working with dilute solutions of highly active enzymes it is frequently necessary to know the amount of protein in the sample. At the present time there are no simple and reliable methods for its determination. Lowry's very well-known method [1] is suitable for determining fairly large amounts of protein — more than 10-15 μg. A number of methods for the quantitative determination of protein in ultramicro amounts is known, but they are all fairly complicated and require additional equipment such as, for example, a scanning fluorimeter [2] or a special membrane [3-6].

We have attempted to find a simpler method of determining small amounts of protein in a sample. As a basis we took a method described in 1969 [7] which permitted the determination of 10-20 μg of protein in a sample. In our modification it is possible to determine 1-10 μg of protein. The method is as follows. A solution of protein is deposited on a sheet of filter paper and is treated with trichloroacetic acid (TCA). Then the sample is stained with a dye, the excess of dye is washed out, the stained complex is eluted, and the optical density of the eluate is measured in a spectrophotometer.

For staining the protein on the paper we tested Coomassie Blue, Amido Black 10B, and Coomassie Brilliant Blue R-250. The first and second gave no advantages whatever over the Lowry method, but the use of Coomassie Brilliant Blue R-250 gave good results. Concentrations of the dye of from 0.1 to 1% had little effect on the density of staining up to 10 μg of protein, but with the higher concentrations of dye (0.5-1%) it was difficult to wash the dye out of the substrate, which considerably increased the adsorption of the blank. The

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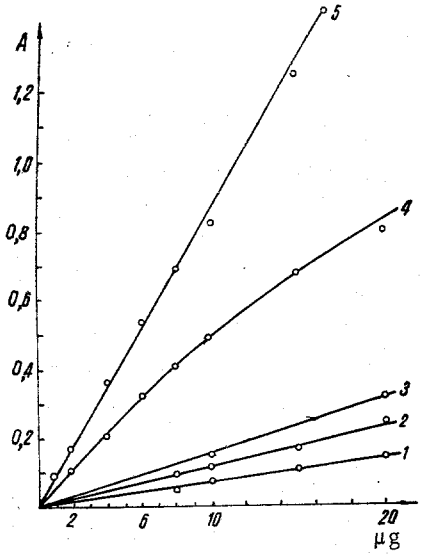

Fig. 1. Dependence of the optical density of an eluate of the stained complex on the amount of protein in the sample: 1) Coomassie Blue, 580 nm; 2) Lowry's method, 750 nm; 3) Amido Black 10B 620 nm; 4) Coomassie Brilliant Blue R-20 in the absence of normal amyl alcohol, 610 nm; 5) the same, with 0.5% of normal amyl alcohol.

optimum concentration is a 0.2% solution of Coomassie Brilliant Blue R-250. The staining of the protein takes place best at an elevated temperature, as has been reported previously [7]. The presence of phosphate and NaCl up to 1 M does not interfere with the determination. Unexpected was the influence of a small amount of normal amyl alcohol on the optical density of the eluted dye–protein complexes: 0.3-0.5% of normal amyl alcohol increased the optical density by a factor of 1.5-2. A further increase in the amount of normal amyl alcohol had no influence on the coloration. The error in the determination of 1 μg of protein was about 4% and for 5-10 μg 2-3%. In the determination of more than 10 μg of protein it is possible to use a 0.1% solution of Amido Black 10B as described previously [5] with the difference that the protein is fixed on the paper with TCA.

The staining of the protein on paper with Coomassie Brilliant Blue R-250 was performed in the following way. The protein solution was deposited on a 1.5 × 2 cm sheet of Whatman No. 1 paper, the spot was dried in a current of hot air and was fixed with 10% TCA, and it was washed with ethanol–ether (1:1) and was dried. The sample was then stained with a 0.2% solution of Coomassie Brilliant Blue R-250 in 7% acetic acid at 50°C for 15 min, and the excess of dye was washed out with 7% acetic acid. The paper was dried and eluted with 1.5 ml of CH_3OH–H_2O–concentrated NH_4OH–n-$C_5H_{11}OH$ (65:34:0.5:0.5 by volume), and the eluate was spectrophotometered at 610 nm.

LITERATURE CITED

1. O. H. Lowry, H. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
2. S. Stein, P. Böhlen, W. Dairman, and S. Udenfriend, Arch. Biochem. Biophys., 155, 213 (1973).
3. T. P. Bennett, Nature, 213, 1131 (1967).
4. H. Kuno and H. K. Kihara, Nature, 215, 974 (1967).
5. W. Schaffner and C. Weissman, Anal. Biochem., 56, 502 (1973).
6. T. Nakao, M. Nakao, and F. Nagai, Anal. Biochem., 55, 358 (1973).
7. S. Bramhall, N. Noack, H. Wu, and J. R. Loewenberg, Anal. Biochem., 31, 146 (1969).