patterns, could be observed. The only exception visible in the plasma pattern of roosters was an additional cloudiness in the post-albumen zone. This observation is in agreement with findings of previous workers who utilized the Tiselius method⁷ for their observations.

The growth of the embryo in the fertilized egg induced considerable alterations in the pattern of egg albumen within the first days of incubation. All zones, particularly the intensively stained heavy base zone, tended to fade. Most resistant among the zones were those which lay in the region of the so-called α - and β -bands. However, after the sixth day, even these became unrecognizable and the pattern disappeared completely.

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¹ Longworth, L. G., Cannan, R. H., and MacInnes, D. A., *J. Amer. Chem. Soc.*, **62**, 2580 (1940). Bain, J. A., and Deutsch, H. F., *J. Biol. Chem.*, **171**, 531 (1947).

² Forsythe, R. H., and Foster, J. F., *J. Biol. Chem.*, **184**, 377 (1950).

³ Poulik, M. D., *Nature*, **180**, 1477 (1957).

⁴ Lush, J. E., *Nature*, **189**, 981 (1961).

⁵ Smithies, O., *Biochem. J.*, **61**, 629 (1955).

⁶ McKinley, W. P., Oliver, W. F., Maw, W. A., and Common, R. H., *Proc. Soc. Exp. Biol. Med.*, **84**, 346 (1953).

⁷ Moore, D. H., *Endocrinol.*, **42**, 38 (1948). Brandt, L. W., Clegg, R. E., and Andrews, A. C., *J. Biol. Chem.*, **191**, 105 (1951).

Progressive Extraction of Proteins

In the course of work on the solubility of wheat proteins, I have developed a method which could also be applied in other fields where a separation according to solubility is desired. This method results from combining the techniques of column chromatography and the lysimetric determinations of soil analysis. Proteins are successively extracted by means of increasing concentrations of the same solvent in water or by a series of different solvents with an increasing ability to dissolve proteins.

The principle is simple and the operations continuous and partly automatic.

10 gm. of wheat flour and 10 gm. of finely ground pumice, passing through a 200- μ sieve, are mixed thoroughly in a mortar; 100 gm. of fine sand (Merck No. 7712) are added and mixed.

A glass tube (25 cm. length, 3.5 cm. diam.), one end of which is drawn down to 0.5 cm. diameter and plugged with glass wool, is filled with a layer of quartz (Merck No. 7530) and a layer of sand each 1.5 cm. thick. Then the mixture as prepared here is added rapidly through a funnel with a hole 2.0 cm. wide, to avoid separation of the sand; the column is covered with another layer of sand and quartz.

After wetting with 50-60-ml. distilled water the column is mounted over a fraction collector. As an example, 150 ml. portions of solvents may be used in the following order: distilled water, isopropyl alcohol 10 per cent (v/v), isopropyl alcohol 30 per cent, 4 per cent lactic acid, 0.5 per cent aqueous potassium hydroxide. Then the column is washed with 50-100 ml. distilled water. The extraction takes 10-60 hr., depending on the particle size of the flour and the type of solvents used.

The solvents separate the proteins into sharply defined fractions. The amount of protein extracted varies between 99 and 101 per cent of the total protein content of the flour.

Instead of using a fraction collector one may collect separately each 150 ml. portion, taking into account that a very small amount of the preceding solvent could be mixed with it and that approximately 50 ml. will remain in the column.

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Inhibition of Microsomal Triphosphopyridine Nucleotide-Cytochrome *c* Reductase

DURING certain work on the distribution and pathways of pyridine nucleotide oxidation in microsomal systems from rat liver, it was found that the oxidation of reduced triphosphopyridine nucleotide (TPNH) was associated with the ribonucleoprotein particle fraction, as well as with microsomes from which the particles were derived. The specific activity of the TPNH oxidation is similar in the microsome and ribonucleoprotein particle fractions. Moreover, the addition of deoxycholate to microsomes or derived particles did not alter TPNH-cytochrome *c* reductase activity. However, it was discovered that the rate of oxidation of TPNH progressively declined with time.

An examination of the time-course of the reaction and of conditions necessary for enzyme activity led to the identification of oxidized TPN as the inhibitory substance. The relationship of TPN concentration in the test system to the velocity of TPNH oxidation is shown in Fig. 1. The severe inhibition of TPNH oxidation with ferri-cytochrome *c* as acceptor*

Fig. 1. TPN inhibition of TPNH oxidation in a ribonucleoprotein fraction derived from rat liver microsomes. Microsomes were prepared by the technique of Sicevitz and Palade (ref. 1), and derived ribonucleoprotein particles sedimented after 3-hr. centrifugation at 105,000*g* after treatment of microsomes with deoxycholate and removal of vesicles. Other conditions as in Table 1.