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It has been shown by Macallum²⁷ that iron is a constant constituent of all chromatin. Mackenzie,²⁸ using the ferrocyanide method and the hæmatoxylin method of Macallum,²⁹ found the Nissl granules to contain iron. Using the hæmatoxylin method, which consists in keeping sections in acid alcohol (sulphuric acid 4, alcohol 100, by volume) for a few hours at 37° C., washing the acid out in alcohol and transferring them to an aqueous solution of hæmatoxylin, one finds the Nissl granules are stained bluish black, which is an indication that they contain iron. Besides the Nissl granules the nucleolus and the oxyphile nuclear substance have the same colour, showing that they also contain iron (Fig. 4). After the sections have been treated with the acid alcohol they may be transferred to acid ferrocyanide solution, when a Prussian blue reaction will be found in the three parts mentioned. The same result is obtained if teased-out cells are baked at 60° C. for several days in a mixture of ammonium sulphide and glycerine according to the method of Macallum, when the Nissl granules, nucleolus and oxyphile nuclear substance turn green, owing to the formation of ferrous sulphide. With any of these methods the Nissl granules are seen in the cell as masses or a reticulum. The appearances obtained by these methods are similar to those obtained by staining with toluidin blue alone, except that the oxyphile nuclear substance is also affected.

Using the test for phosphorus as described by Macallum,³⁰ the Nissl granules, nucleolus and oxyphile nuclear substance give a marked reaction for phosphorus, while the intergranular spongioplasm gives a faint reaction. (Fig. 3). For the purposes of this test, material that has been fixed in alcohol is extracted in a Soxhlet apparatus and imbedded in paraffin. Sections fixed to the slide are washed several times in distilled water to insure the absence of all alcohol, and then transferred to a solution of ammonium molybdate in nitric acid. After sections have been in this solution for some time they are brought into a solution of phenylhydrazin hydrochloride which reduces the phospho-molybdate to a greenish oxide of molybdenum but does not reduce the molybdate itself. Sections treated for a few minutes in the molybdate solution show little or no phosphorus. It is necessary to leave the sections in the molybdate solution for several hours in order to bring out clearly a reaction in the cell. After treating with phenylhydrazin hydrochloride the

²⁷ Macallum, A. B., "On the Distribution of Assimilated Iron Compounds, other than Hæmoglobin and Hæmatins, in Animal and Vegetable Cells," *Quarterly Journal of Microscopical Science*, Vol. XXXVIII, p. 175, 1895.

²⁸ Mackenzie, I. C.

²⁹ Macallum, A. B., "A New Method of Distinguishing between Organic and Inorganic Compounds of Iron," *Journal of Physiology*, XXII, p. 92, 1897.

³⁰ Macallum, I. C.