SUMMARY

In chapter 1 Nissl’s original staining technique for Nissl bodies is described, moreover the further development of this staining technique. At the same time is mentioned that the recently developed methods for cytological identification of nucleic acids can be employed for the staining of Nissl bodies.

It is pointed out that the first real advance since Nissl’s original method was Einarson’s introduction of the gallocyanin-chromalum technique in 1932. This new staining technique made it possible for Einarson (1933-35) to demonstrate that the Nissl substance contained a basophilic chromatin substance which no doubt derived from the nucleus. Thus Einarson confirmed Scott’s hypothesis of 1899.

Later on the results achieved by the Casperson ultraviolet microspectrophotometric technique have confirmed and amplified Einarson’s previous results.

At the end of the chapter a method of the staining of nucleic acid based on its content of pentose is mentioned, as well as a nucleic acid staining method based on the use of the dye acridine orange in connection with fluorescence microscopy.

Chapter 2 gives an account of Einarson’s gallocyanin-chromalum technique. It is emphasized that this staining method is progressive and highly specific of nucleic acids when applied within the range of pH 0.8-1.75.

The specificity of the gallocyanin-chromalum technique as to nucleic acids is proved by the complete identity between the results achieved by this staining method and results achieved by ultraviolet microscopy at 2600 Å. The specificity of this staining method is also confirmed by tests with ribonuclease.

At the end of the chapter the acridine orange staining technique and the almost classic Feulgen technique are described.

Chapter 3 contains a discussion based on the literature of the relation between the Nissl picture of the fixed cell and the conditions of the living cell. At this time it must be allowed to consider the following statements as sufficiently proved:

The Nissl substance exists as limited aggregates in the cytoplasm of the living nerve cell. The Nissl picture found in a nerve cell after an adequate and not too strong fixation is to a very high degree identical with the one found in the living cell. The fixation causes no distinct changes in the structure and distribution of the Nissl substance.

These statements must be considered to be valid as to quality. In case it is a question of quantity the possibility may be anticipated that a certain loss of nucleo-proteins to the fixing fluid takes place when fixing the cells.

Chapters 4 and 5 contain my own investigations. Chapter 4 is opened by an account of the applied preparation technique. It is emphasized that only Carnoy’s fixative has been employed, as this fixative according to the available results is the chemical fixative that causes the slightest changes in the native state of the nucleoproteins.
At the end of chapter 4 is an account based on the literature on the conditions of employing ribonuclease in cytochemical investigations. It is concluded that the ribonuclease test when under the right conditions must be regarded as completely safe and specific provided that a crystalline, protease-free nuclease preparation is employed.

Chapter 4 is finished by emphasizing that is must be considered unlikely that the employed preparation technique should involve any substantial loss of ribonucleoprotein from the Nissl substance of the nerve cells prior to the staining experiments.

Chapter 5 describes the very staining experiments; these are arranged in 16 series of experiments. Each series of experiments is opened by a formulation of the point to be illustrated by the experiment. Then a complete account of the employed technique is given; the achieved results are described and the conclusions are drawn.

Chapter 6 contains a discussion of the achieved results. The conclusions arrived at in chapter 5 are summed up in a series of “statements” formulated in 11 points. This is followed by a brief summary of the most important theories on the use of basic and acid dyes in the histological technique.

By comparing the current opinions of the mechanism of the histological staining with basic and acid dyes with the statements put forward at the beginning of the chapter, the following “staining theory” is arrived at:

The basic dyes belonging to the azine, oxazine, and thiazine dyes, and to the pyronin group of the xanthene dyes stain the Nissl substance and the nuclear chromatin at pH lower than 2. This staining is due to the content of nucleic acid within the substances. This nucleic acid staining comes into being by a salty bond between the phosphoric acid of the nucleic acids and the applied dye. The bond between the phosphoric acid of the nucleic acids and the dye is most sparingly soluble.

At the end of the chapter it is demonstrated that the Nissl substance contains a strongly acid protein which can be stained by the employed basic dyes even at a pH from about 3.0 to 2.60. As it cannot be excluded that this “strongly acid” protein is associated with other types of cytoplasmic ribonucleic acid, a qualitative and quantitative staining of the cytoplasmic ribonucleic acid and the Nissl substance must be based on staining methods employed at a pH lower than 2, but higher than 1. Moreover an adequately long staining time (24-48 hours) is necessary.