Histochemical Localization of Ribonucleoproteins by Alkaline Hydrolysis.* (18963)

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Several methods for the histochemical identification and localization of ribonucleic acid (RNA) and its differentiation from desoxyribonucleic acid (DNA) are now available. They fall into 3 categories: (1) The utilization of the specific ultraviolet absorption of nucleoproteins as described by Caspersson (1,2); (2) The direct specific staining of nucleoproteins by technics such as the Feulgen-Schiff method for the detection of DNA and Turchini's(3) stain for the detection of both DNA and RNA. (3) The use of substances which depolymerize or alter the nucleic acids so that their basophilic staining properties are lost.

Caspersson's ultraviolet absorption methods have been discussed extensively. They need not be considered further in this connection. The Feulgen-Schiff reaction is the most widely accepted method for the demonstration of DNA. Turchini's technic, which includes the use of the dyestuffs 9-phenyl (or methyl) -2,6,7-trihydroxy-3-fluorone, stains RNA yellow-pink and DNA blue-violet. This method has not been generally accepted. Of the methods in the 3rd category, Brachet's(4) ribonuclease test has been used most widely. The cost of ribonuclease, however, makes this method too expensive for routine use in most laboratories. The activity of the commercially prepared enzyme furthermore is not constant. Other methods which have been reported include the trichloroacetic acid method of Pollister and Ris(5) and the perchloric acid method of Sulkin and Kuntz(6). The former method involves the use of high temperatures which may be objectionable. The latter method yields the most satisfactory results only when frozen sections are used or when small blocks of tissue are treated with the reagent.

In the course of an investigation in which various staining methods have been used following the application of McManus and Cason's(7) acetylation method, it has been observed that a potassium hydroxide bath, such as that used by McManus, abolishes the basophilic staining reaction of the chromidial substance and the nucleoli, in nerve cells without altering the basophilic staining reactions of the nuclei. The present paper embodies the results of a further investigation of this phenomenon.

Methods. Sections of autonomic ganglia of dogs, cats and man were treated with the acetic anhydride-pyridine mixture used by McManus for 40 minutes. This was followed by a 0.1 N KOH bath for 45 minutes. Other sections were placed in a 0.1 N KOH bath for 45 minutes without having been treated with the acetic anhydride-pyridine mixture. The sections were then stained with 0.25% toluidin blue, 1% toluidin blue, Harris hematoxylin and eosin or Mallory's methyline blue-phloxine stain.

The sections treated with the acetic anhydride-pyridine mixture showed no alterations in their basophilic staining properties. Those treated with this reagent followed by a bath of KOH or with KOH alone showed alterations in the basophilic properties, indicating that the 0.1 N KOH is the factor responsible for abolition of the basophilic staining reaction.

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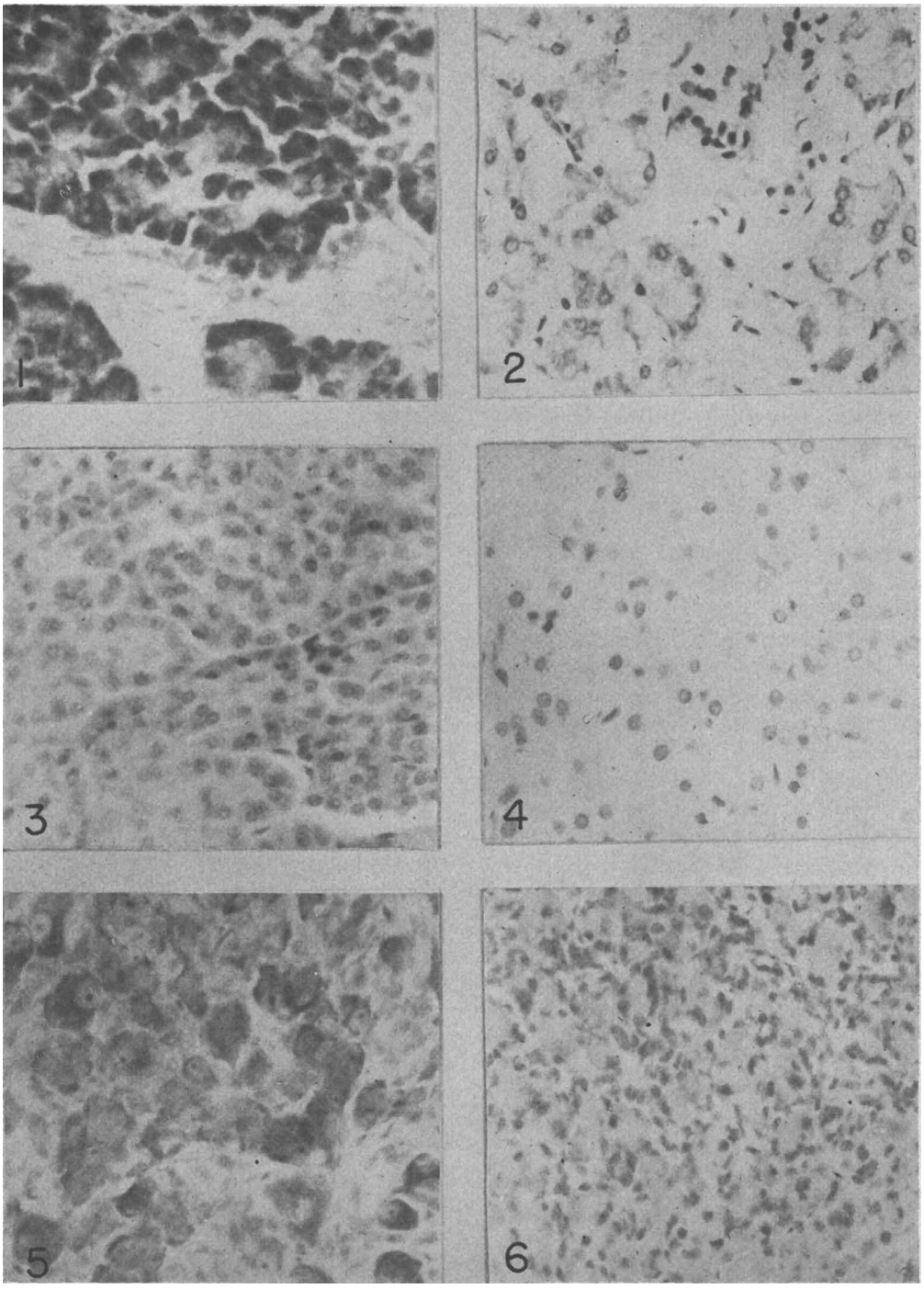

FIG. 1. Section of human parotid gland stained with toluidin blue, ocular 10×, obj. 8 mm.

FIG. 2. Section of pancreas from cat stained with toluidin blue, ocular 10×, obj. 8 mm.

FIG. 3. Section of human parotid from same series of sections as Fig. 1 which was treated with 0.1 N KOH for 45 min preceding the toluidin blue stain, ocular 10×, obj. 8 mm.

FIG. 4. Section of pancreas of cat from same series of sections as Fig. 2 which was treated with 0.1 N KOH for 45 min preceding the toluidin blue stain, ocular 10×, obj. 8 mm.

FIG. 5. Section of autonomic ganglia from 6-week-old dog stained with toluidin blue, ocular 10×, obj. 8 mm.

FIG. 6. Section similar to that in Fig. 5 which had been treated with 0.1 N KOH prior to staining with toluidin blue.

The following procedure has been used successfully on autonomic ganglia of dogs, cats and man, parotid glands of man and pancreas of cats and rats. The tissues were fixed in 10% formalin, Zenker's fixative or 90% alcohol, dehydrated, imbedded and sectioned in the routine manner. Following removal of the paraffin the sections were taken through a degrading series of alcohols and placed in water. One-half of each series of sections was placed in 0.1 N KOH for 45 minutes at room temperature and then thoroughly washed in tap water. The other half of each series of sections was allowed to remain in water during the same time. Both the control and the alkaline treated sections were then stained together in 0.25% or 1% toluidin blue, Harris hematoxylin and eosin or methyline blue-phloxine.

The cytoplasm of the parotid gland cells (Fig. 1) and the acinar cells of the pancreas (Fig. 2) is normally basophilic. Treatment of the sections with ribonuclease prior to staining inhibits the basophilic staining reaction. This indicates that the cytoplasmic basophilia is due to the presence of ribonucleic acid. In all instances, sections of the parotid gland (Fig. 3) and the pancreas (Fig. 4) that were treated with mild alkaline hydrolysis showed no basophilic staining in the cytoplasm. The DNA of the nuclei remained unaltered (Fig. 3 and 4). The basophilic staining reaction of the chromidial substance and the nucleoli of autonomic ganglion cells (Fig. 5), as has been previously reported by Caspersson(1), Gersh and Bodian(8), and others, is due to the presence of RNA. This reaction was inhibited by treatment of the sections with 0.1 N KOH (Fig. 6).

Conclusions. The alkaline hydrolysis method described above for the histochemical localization of RNA and its differentiation from DNA is a simple and convenient method for general use. It is apparently also as reliable as any procedure previously reported.

The differential hydrolysis of RNA and DNA probably is due to the position of the phosphoric acid residues connecting the nucleotides. Because of the greater resistance of the DNA to alkaline hydrolysis as compared to the resistance of RNA, Levene and Tipson(9) have advanced the opinion that the hydroxyls of carbons 3 and 5 of neighboring desoxyribose units of DNA are connected. In RNA, the hydroxyls of carbons 2 and 3 of the ribose units probably are involved. The data obtained in the present study afford further evidence of the resistance of DNA to mild alkaline hydrolysis.

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Comparative Effect of Tryptophan, Niacin, and Nicotinamide in Forming Liver Pyridine Nucleotides.* (18964)

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The ability of tryptophan to replace niacin in the diet of the rat was first demonstrated by Krehl *et al.*(1), who used growth as a criterion of activity. More recent studies in our laboratory indicate that tryptophan and niacin, when incorporated in the ration in equimolar amounts, except at very high levels,

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