Further Cytochemical Studies on the Perichromatin Granules*

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Summary. The perichromatin granules were studied in hepatocytes of experimental rats injected with cycloheximide because the increased number of these nuclear components after such treatment facilitated their cytochemical investigation. Most perichromatin granules were sensitive to the digestion with pepsin and ribonuclease. In contrast, small population of perichromatin granules was resistant to such digestion under conditions which remove known RNA containing components such as ribosomes, nucleolar RNP components and interchromatin granules. The size of these resistant perichromatin granules was reduced and they consisted of filaments the width of which was similar to that of filaments in the chromatin. Moreover, a small population of perichromatin granules was sensitive to the digestion with pepsin and deoxyribonuclease. The size of these granules was only slightly reduced. All these observations indicate that most perichromatin granules contain the RNA and some the DNA. A possibility also exists that the perichromatin granules might contain both RNA and DNA but in various proportions. In addition, partial digestion with pepsin followed by a complete digestion with ribonuclease and deoxyribonuclease removed perichromatin granules as well as other nucleoprotein structures. On the other hand, such digestion facilitated the visualization of the nuclear and cytoplasmic skeleton (matrix) in situ.

Introduction

Since their first descriptions, perichromatin granules are a subject of numerous reports and discussions in the cytological literature (Bernhard, 1969; Bouteille et al., 1974; Monneron and Bernhard, 1969; Puvion and Bernhard, 1975; Simard et al., 1978; Smetana, 1977; Vasquez-Nin and Bernhard, 1971; Watson, 1962). However, nor their composition nor their function have been satisfactorily

* Dedicated to the memory of Dr. W. Bernhard
clarified and still are a subject of discussions which are frequently controversial (Bouteille et al., 1974; Monneron and Bernhard, 1969; Petrov and Sekeris, 1971; Smetana, 1977; Vasquez-Nin and Bernhard, 1971; Watson, 1962). On the other hand, the ultrastructural cytochemistry indicated that the perichromatin granules contain the RNA and according to few studies also the DNA (Bernhard, 1969; Bouteille et al., 1974; Monneron and Bernhard, 1969; Puvion and Bernhard, 1975; Smetana, 1977; Vasquez-Nin and Bernhard, 1971; Watson, 1962).

To provide more information on the cytochemistry of perichromatin granules, these nuclear components were studied in hepatocytes of experimental rats which were treated with cycloheximide. The cycloheximide treated rats represent a very convenient model for such studies since this drug increase substantially the number of perichromatin granules in hepatocytes (Daskal et al., 1975).

The results clearly indicated that the digestion of hepatocytes with pepsin followed by ribonuclease removed about 90% of perichromatin granules. The size of remaining granules was reduced in comparison with undigested particles or particles digested only with pepsin. The digestion of hepatocytes with pepsin followed by deoxyribonuclease also decreased the number of perichromatin granules but much less. The size of these granules was only slightly smaller than in the control specimens. After partial digestion with pepsin followed by complete digestion with ribonuclease and then with deoxyribonuclease perichromatin granules were not observed. Such digestion, however, facilitated the visualization of the nuclear skeleton.

Material and Methods

Three male Holtzman rats (200–250 g) were injected intraperitoneally with 200 mg/kg of cycloheximide (Calbiochem) dissolved in 1 ml of 30% ethanol (Daskal et al., 1975). Six hours later the rats were decapitated and small liver slices were fixed either in 2.5% glutaraldehyde in phosphate buffer (pH 7.4) for 1 h or in buffered formaldehyde (see blow) for enzymatic extraction procedures. All specimens (including those after enzymatic extractions) were dehydrated in ethanol containing uranyl acetate and embedded in Epon-Araldite mixture (Mollenhauer, 1964; Smetana, 1977; Smetana et al., 1968). Ultrathin sections cut with a Reichert OM U2 ultramicrotome were stained with uranyl acetate followed by lead citrate (Venable and Coggeshall, 1965) and observed with a Philips 300 electron microscope.

The enzymatic extraction procedures were carried out on small liver slices after a very short fixation (10 min) in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C and terminated by treatment with 5% trichloracetic acid at the same temperature for 15 min. The following digestion procedures were used: (a) partial digestion with pepsin (0.01 mg/ml ml of 0.1 N HCl) for 10–15 min; (b) partial digestion with pepsin for 10 min followed by a wash (ice-cold) in phosphate buffer (0.1 M, pH 7.4) and digestion with ribonuclease (1–2 mg/l ml of acetate buffer at pH 5 was heated at 80°C for 10 min to remove deoxyribonuclease activity and then the pH was adjusted to 6.5 with 0.01 N NaOH, see Smetana et al., 1968) for 2 h; (c) partial digestion with pepsin followed by a wash in ice-cold phosphate buffer and digestion with deoxyribonuclease (1–2 mg/l ml of 0.003 M magnesium acetate) for 2 h; (d) partial digestion with pepsin for 10 min followed by a wash in ice-cold phosphate buffer, digestion with ribonuclease for 2 h, ice-cold wash in phosphate buffer and then digestion with deoxyribonuclease for 2 h. All specimens were digested at 37°C and for details see ref. Smetana et al., 1968. The enzymes used in the present study were obtained from Worthington Biochemical Corporation, Freehold, N.J. and Sigma, St. Louis, Mo. For controls of enzymatic extractions aliquots of digested specimens were incubated under the same conditions but without the presence of enzymes.