OBSERVATIONS ON A GRANULE ASSOCIATED WITH CHROMATIN IN THE NUCLEI OF CELLS OF RAT AND MOUSE

MICHAEL L. WATSON. From the Departments of Pathology and Radiation Biology of The University of Rochester School of Medicine and Dentistry, Rochester, New York

The use of indium in this laboratory as an electron stain for nucleic acids has revealed in mammalian nuclei a granule which we believe to be hitherto unreported. The purpose of this note is to define and describe this entity and to indicate its presence in the nuclei of at least some tissues of the rat and mouse.

METHODS

Most tissues described here were processed according to the indium procedure and embedded in cross-linked methacrylate (1). The fixative used was slightly modified from the original procedure in that it contained, in addition to 10 per cent acrolein, 0.3 M sucrose and 0.002 M $MgCl_2$ (2). The pH was

adjusted to about 7.5 by addition of NaOH immediately before use. Tissues examined came either from CFW (Carworth) mice or Wistar-Rochester stock rats.

In some cases, instead of the indium procedure, following fixation tissue blocks were stained in fixative to which had been added about 1 per cent uranyl acetate at a pH in the range of 3.5 to 4.0. They were then washed briefly in fixative without the uranyl, dehydrated in acetone, and embedded in Vestopal. Some blocks were also stained instead with alcoholic uranyl acetate and embedded in Vestopal.

All methacrylate-embedded tissue sections were sandwiched between carbon and formvar to minimize loss of embedding material in the electron microscope. A Siemens Elmiskop I was used provided with a 50-micron objective aperture and operating at 80 kv.

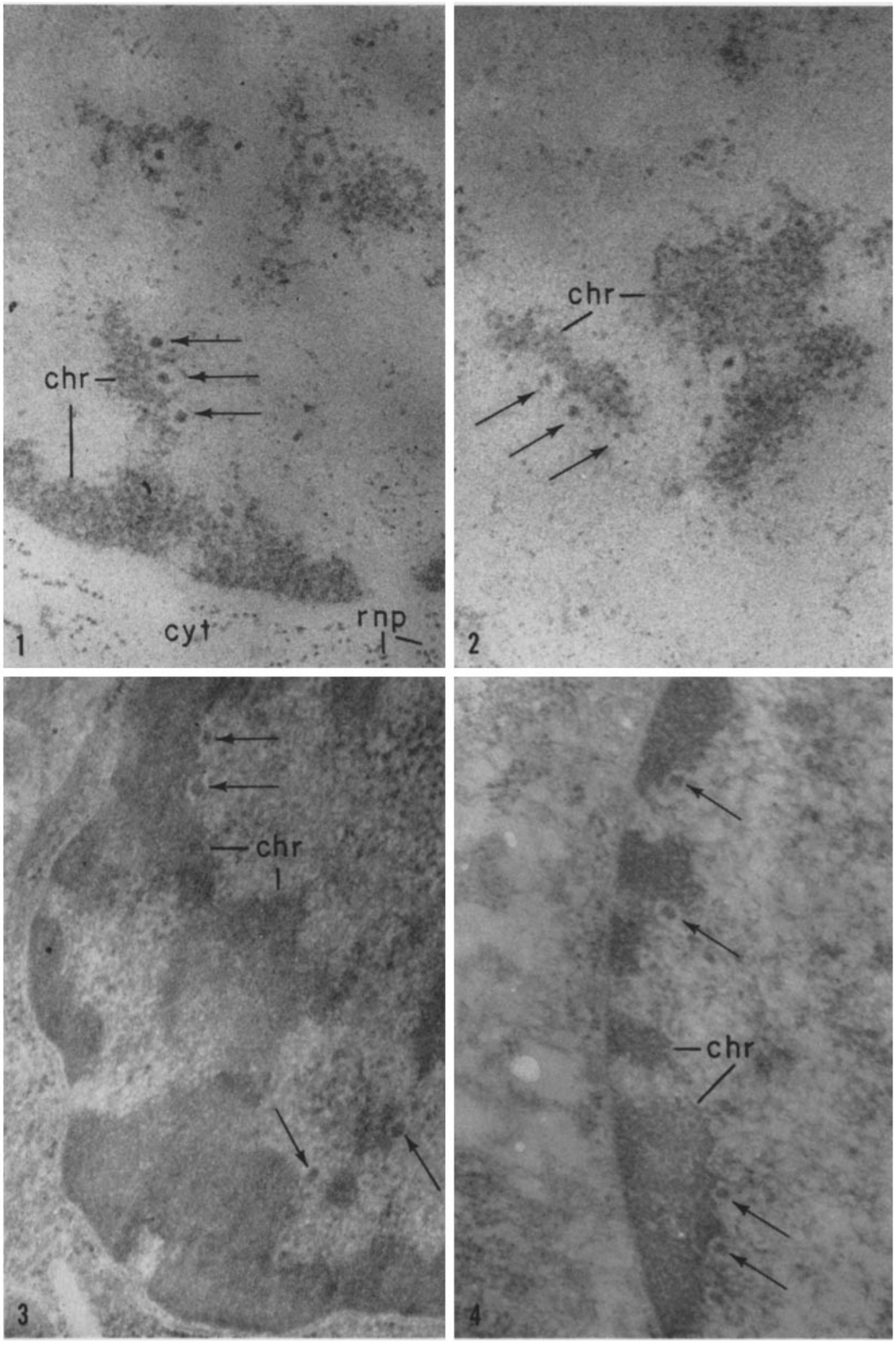
RESULTS AND DISCUSSION

We have previously described, in the nuclei of tissues fixed with acrolein and stained with indium, masses of material which tend in interphase to concentrate at the surface of the nucleus and around the nucleolus. Such masses are disposed in the manner of Feulgen-positive material seen in thick sections of the same tissue blocks and may tentatively be considered as containing deoxyribonucleic acid (DNA), hence as homologous with chromatin. This identification is strengthened by observations to be reported elsewhere of tissue treated with cold perchloric acid and subsequently stained with indium. In these studies (3) it was found that all but a small percentage of ribonucleic acid (RNA) was removed, but that DNA and in particular the above-mentioned masses of material remained and were stainable with indium. The granule here under consideration is usually found at or near the surface of chromatin masses and will, therefore, be referred to as the *perichromatin granule*. The granule is about 300 A in diameter and is spaced from surrounding chromatin by a clear zone or mantle which stains very little or not at all and has a thickness of about 250 A. The entire assembly, then, of granule and mantle has an overall diameter of about 750 A (Figs. 1 and 2). In general, granules appear to have a density slightly greater than that of the neighboring chromatin. The density of some, however, is less and ranges down to a level which is difficult to detect (Fig. 2). This is, in part, due to only partial inclusion of a granule in the section, but in some cases may result from a lower concentration of stainable material in the granule.

The concentration of perichromatin granules per nucleus is such that in a section of average thickness of a rat hepatic nucleus the number of granules is in the range of 5 to 20. If such a section is 500 A thick and the nucleus 7 microns in diameter it might contain between 500 and 2000 granules. Except that they are usually located on the surface of clumps of chromatin, granules show no preferential location within the nucleus. They range in position from the nuclear envelope (Figs. 1 and 4) to the nucleolus (Fig. 6).

The perichromatin granule may be visualized not only by the indium procedure, but also in acrolein-fixed tissues stained in the block with aqueous uranyl acetate (pH 3.5 to 4) or with alcoholic uranyl acetate (Figs. 3 and 4). In general, it is easily identified only in cells in which chromatin is clumped. The quality of fixation with acrolein varies considerably from the surface of tissue blocks to the interior. The chromatin of hepatic nuclei near the surface of blocks of liver, for example, is not clumped but rather evenly dispersed. In such nuclei the perichromatin granule cannot be identified. Nuclei of cells other than hepatic cells may not contain dispersed but, instead, clumped chromatin. In such cells perichromatin granules are found.

It is possible to stain sections of tissues processed by the indium method with saturated aqueous uranyl acetate. The contrast of nucleic acid–containing regions is considerably enhanced by uranyl over what is obtained with indium alone. General non-specific staining also appears despite the fact that the tissue has been subjected to reduction and acetylation procedures (*cf.* cytoplasm in Fig. 5). Perichromatin granules are seen clearly with uranyl staining (Fig. 6). In addition, clusters of similar granules but somewhat variable in size are evident (Figs. 7 and 8). Such clusters are not readily apparent with indium alone, probably due to the lower general contrast. They may, however, be elements which do not contain nucleic acid and are non-specifically stained by the uranyl, or they may contain small amounts of nucleic acid and thus be weakly stained by indium. If the latter is true, they would seem to represent different stages in the formation or dissolution of perichromatin granules. It is hoped that complete extraction of nucleic acid will settle this point.



Perichromatin granules are not recognizable with confidence in tissues fixed with either potassium permanganate or osmium tetroxide. This may be due to a difference in the way nucleic acids are preserved by these methods or to contrast conditions which mask them.

Perichromatin granules, in addition to being seen in hepatic cells, have been found in a variety of other tissues of the rat (Figs. 3, 9, and 10) including eosinophils, endothelial cells, striated muscle, mast cells, and convoluted tubule cells. The granule has also been found in cells of mice (Figs. 1, 2, and 6). A preliminary examination of the livers of axolotl and *Xenopus laevis* failed to reveal the presence of perichromatin granules.

Treatment with cold perchloric acid does not noticeably affect the morphology of cytoplasmic structures except for the removal of RNP particles, but there are changes in the fine structure of DNA which are difficult to assess (2). It is possible after perchloric acid treatment to find perichromatin granules, but they are not as distinct as in the unextracted tissues (Figs. 11 and 12), nor do they stain so heavily relative to chromatin. It appears that at least some of the granules resist perchloric acid and may, therefore, contain DNA. Decrease in the intensity of staining may be due to the presence of RNA as well. An apparent decrease in the concentration of particles on perchloric acid extraction may be due to distortion beyond recognition or perhaps to a content only of RNA and not DNA.

The possibility that perichromatin granules contain nucleic acid rests at present on their stainability with indium and uranyl. The indium procedure may be expected to result in staining of elements having a high percentage of strongly acidic groups, such as phosphate, carboxyl, and perhaps sulfate, which must be in a form resistant to extraction by aqueous fixative and various solvents. The commonest substance in cells which meets these requirements appears to be nucleic acid, but we can present no convincing argument against the presence of other suitable substances in the nucleus responsible for staining of perichromatin granules. For the present, however, we favor the view that they do indeed contain nucleic acid.

The shape and size of this granule and the possibility that it contains DNA and perhaps

Explanation of Figures

Magnification of all figures is 60,000.

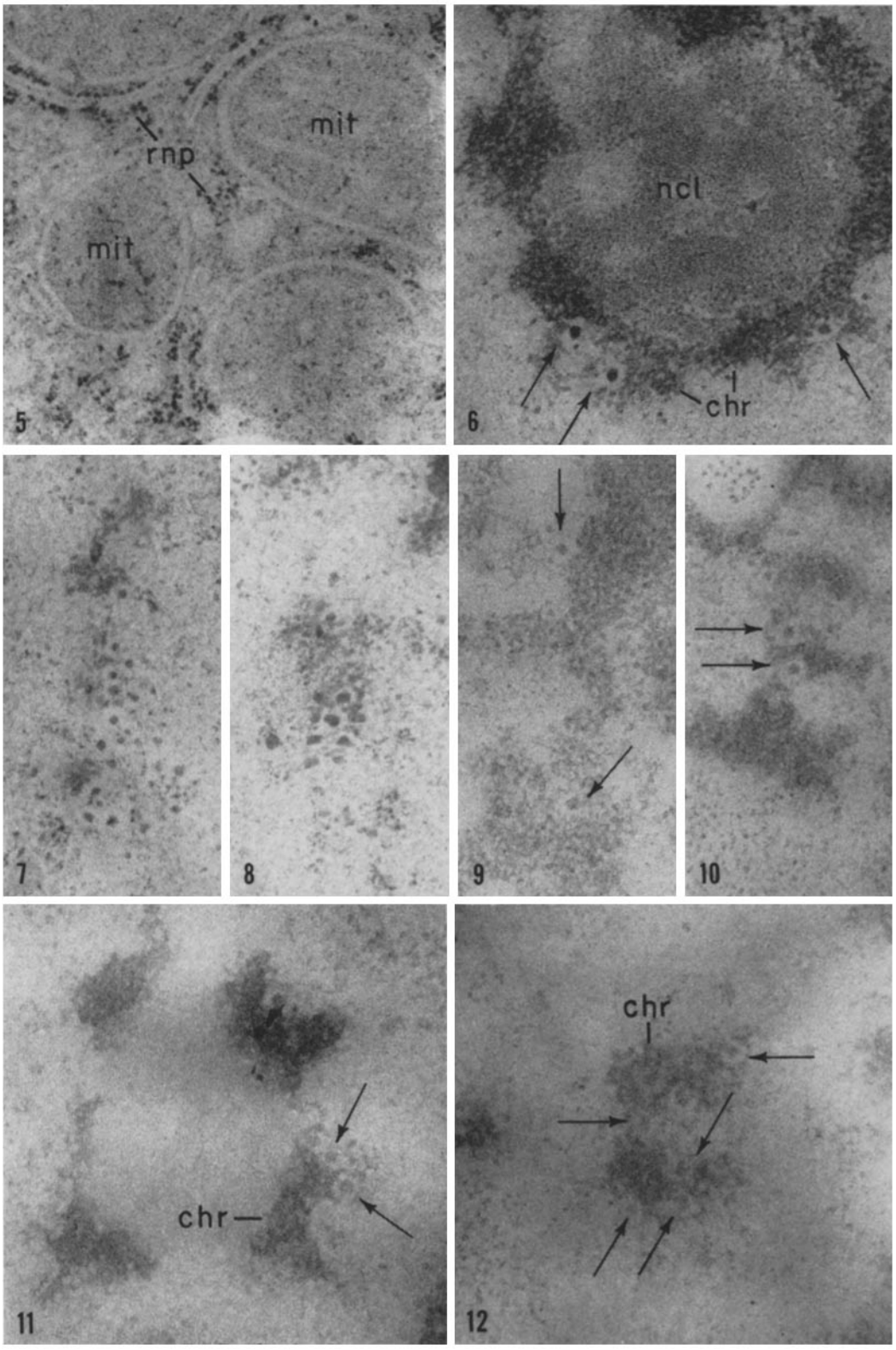
Symbols are as follows: Arrows indicate perichromatin granules; *chr* = chromatin; *cyt* = cytoplasm; *mit* = mitochondrion; *ncl* = nucleolus; *rnp* = ribonucleoprotein particles.

FIGURES 1 AND 2

Sections of mouse hepatic cells following indium treatment. Perichromatin granules are seen about 300 A in diameter surrounded by a clear zone with an over-all diameter of about 750 A. The granules are located primarily on the surface of clumps of indium-staining material identified as chromatin. The granules appear on the average to be stained slightly more strongly than the neighboring chromatin. Some granules of smaller size and perhaps lower density are seen particularly in Fig. 2. The granules may be compared in size and appearance with RNP particles in the cytoplasm in Fig. 1.

FIGURES 3 AND 4

Cells in the liver of rat after staining with alcoholic uranyl acetate and embedding in Vestopal. Perichromatin granules are seen in both micrographs in association with clumps of chromatin. In addition to staining chromatin, uranyl under these conditions, in the paler regions of the nucleus, stains material which does not stain with indium and which is presumed to be protein. The perichromatin granules have about the same density as the neighboring chromatin, perhaps due to staining of protein as well as nucleic acid in the chromatin. Fig. 3 shows an endothelial cell nucleus and Fig. 4, an hepatic cell nucleus.



RNA suggest that it may be a virus. The arguments, however, either for or against this interpretation are at present weak. In favor, the shape and size and possible content of DNA have been mentioned. Against its being a virus the arguments are slightly more involved. With our present limited knowledge of the morphology of the nucleus we could hardly be surprised to find a non-viral accumulation of DNA of this sort. The clear zone surrounding the particle may be present in the living cell or it may represent shrinkage, arising during preparation, of either (or both) the chromatin or granule from its neighbor. It has not been possible to stain in any way a membranous or proteinaceous coat around the particle. We would expect such a coat to be visible in osmic preparations since viral coats are well preserved by such treatment, but it has not been seen. Finally, we note that perichromatin granules can be found in a number of different cells of the rat. In order to be viruses, then, they must be able to infect at least that many different cells of the rat as well as cells of the mouse. Thus, they must represent either a rather versatile virus or several different viruses. Further investigation with a number of stains and species of animals may help to settle this point.

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FIGURES 5 THROUGH 8

Sections of rat hepatic cells following indium treatment and stained after sectioning with saturated aqueous uranyl acetate at pH 3.5 to 4.

Fig. 5 shows the cytoplasm with non-specific staining of mitochondrial and other regions. The density of RNP particles indicates relatively greater staining of nucleic acids.

Fig. 6 shows a nucleolus enveloped in chromatin with associated perichromatin granules.

Figs. 7 and 8 show unidentified granules in the nucleus. Some of these may be perichromatin granules, but such closely spaced aggregations are rarely, if ever, encountered with indium staining alone.

FIGURE 9

Section through the nucleus of a striated muscle cell in rat tongue, stained with indium, showing perichromatin granules.

FIGURE 10

Section through a mast cell nucleus in rat tongue, stained with indium, showing perichromatin granules.

FIGURES 11 AND 12

Sections through rat hepatic nuclei after treatment for 16 hours in cold 10 per cent perchloric acid followed by indium staining. Although these small fields do not show cytoplasm or nucleolus, RNP particles and similar appearing particles in the nucleolus have been removed. Perichromatin granules have survived the perchloric acid extraction. Relative to the chromatin they stain less intensely with indium after than they do without extraction (*cf.* Figs. 1 and 2).