Effect of Quinacrine on Nuclear Structure and RNA Synthesis in Cultured Rat Hepatocytes

Elizabeth H. Leduc, Wilhelm Bernhard, Annie Viron, John Fain, and Edmond Puvion

ABSTRACT

The effects of quinacrine, an antimetabolite which intercalates into DNA, on the ultrastructure of interphase nuclei and on RNA turnover were studied in primary cultures of rat hepatocytes. Procedures included ultrastructural cytochemical staining for ribonucleoprotein and DNA, autoradiography, and measurement of labeled uridine uptake and incorporation. Addition to the culture medium of a nontoxic dose (10 μM for 30 min) reduces the net accumulation of labeled uridine in RNA. This involves first heterogeneous RNA and then ribosomal RNA since their structural precursors, interchromatin fibrils and nucleolar fibrils, respectively, diminish in that order. Intranuclear chromatin retracts, and perinuclear chromatin becomes unusually condensed. A toxic dose (50 μM for 30 min) produces greater inhibition of tritiated uridine incorporation in RNA. This precedes and is not due to a drop in uridine uptake into the cells. Toxic doses produce unusually large clusters of interchromatin granules which are embedded in an unusual dense material which stains positively for ribonucleoprotein. Three regions of the chromatin are altered. (a) Perinuclear condensed chromatin retracts from the nuclear envelope, remaining attached by short DNA-containing bridges. (b) The normally dispersed nucleoplasmic chromatin condenses into a stainable network which retracts centrifugally. (c) Perinucleolar chromatin becomes a network of small highly condensed masses or bands interconnected by fibrils which are either decondensed or stretched. These alterations in chromatin structure probably form the basis of quinacrine-impaired nuclear metabolism.

INTRODUCTION

Aminoacridine antimetabolites including quinacrine bind to DNA at specific sites rich in adenine and thymine bases (10). In isolated DNA, the aminoacridine molecules become intercalated between base pairs and cause a slight unwinding and lengthening of the DNA molecules, a deformation that has been postulated to be related to the in vivo growth-inhibiting properties of these compounds (8, 29). Quinacrine has been used extensively for the control of viral, bacterial, and protozoan infectious agents, especially the malarial plasmodia (23, 29). It also has been reported to have antineoplastic effects (8). Together with the related substituted 4-aminoquinolines, chloroquine and hydroxychloroquine, it is used in the management of connective tissue diseases, particularly the proliferative pannus of rheumatoid arthritis (16) and the skin lesions of lupus erythematosus (15). The mechanism of action of these compounds is not known, but it may involve, in part, an inhibition of lysosomal function. In this investigation, we present some of the dose-related effects of quinacrine on interphase nuclei.

The aminoacridines have been used as a means of studying the structure of DNA and chromatin. The intercalation of ethidium bromide was used to analyze DNA supercoiling (12), and the binding of quinacrine to chromosomes (Q-banding) has been related to the condensation of chromatin as well as to its base composition (10). Three related acridines have been shown to induce unique alterations in the ultrastructure of components of the interphase nucleus. These included the effects of proflavin on cultured rat embryo cells (33), quinacrine in L strain mouse fibroblasts (20), and chloroquine in Al-I lymphoma and Cloudman S91 melanoma cells (2).

We have initiated studies on the effects of intercalating compounds on the structure and metabolism of the interphase cell nucleus. In this paper, we present the effects of quinacrine at various dose levels and time intervals on cell survival, the ultrastructure of the interphase nucleus, and the incorporation of radioactive uridine into nuclear RNP.4 Primary cultures of isolated rat hepatocytes were used as models of nonreplicating cells to compare the nuclear alterations induced by intercalating compounds with those produced by other types of active agents (7, 19).

MATERIALS AND METHODS

Cell Cultures. Hepatocytes were isolated from 150-g WAG rats by standard procedures (30). The cells were suspended in Eagle’s minimal essential medium containing 10% fetal calf serum, 7.5 × 10^5 cells/ml, 3 ml/Falcon flask, and incubated at 37° in an atmosphere containing 5% CO₂. They became attached to the plastic substrate after 1 hr, when the medium containing some dead cells was replaced with fresh medium.

Treatment. Treatments with quinacrine were carried out after 24 h of cell incubation in the above medium. Quinacrine·2HCl (Serva, Heidelberg, Germany) was added to the culture medium to attain final concentrations of 10, 50, 100, or 500 μM, which represent a range of doses used in other types of experiments (25). The cells were prepared for examination after 15 min to 2 hr but occasionally after shorter or longer periods as indicated in "Results."

Cell Survival. Viability was tested by trypan blue exclusion in cultures treated with 10 or 50 μM quinacrine for 30 min and then incubated in drug-free medium for 24 hr. The dye was added directly to the medium in the culture flasks to attain a

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2 To whom requests for reprints should be addressed, at Box 1955, Brown University, Providence, R. I. 02912.
3 Deceased.
4 The abbreviations used are: RNP, ribonucleoprotein; TCA, trichloroacetic acid.
final concentration of 0.5%. The surviving cells were counted in 3 fields at ×80 with the aid of an ocular grid. The number of cells in 100 squares of the grid was extrapolated to give the total number of attached viable cells on the 25 sq cm area of the culture flask.

**RNA Synthesis.** The incorporation of radioactive uridine into the acid-soluble and -insoluble components of the cells was measured in the presence of either 10 or 50 μCi [5-3H]uridine per ml (specific activity, 28 Ci/mmol; CEA, Saclay, France) in the complete medium. Routine TCA precipitation and scintillation counting procedures were followed. To determine whether the presence of quinacrine in scintillation tubes would cause quenching and reduce counting efficiency, cell cultures labeled previously with [5-3H]uridine for 45 min were exposed to 10, 50, or 500 μM quinacrine for 2 min and then prepared for counting. No quenching was detected. The details of individual experiments are described under "Results."

**Electron Microscopy.** The cells were fixed with 1.6% glutaraldehyde in phosphate buffer at pH 7.3 for 45 min. An occasional sample was postfixed in 1% OsO₄. Structure was revealed by conventional uranyl acetate-lead citrate staining of Epon sections. Preferential staining of RNP was carried out by the procedure of Bernhard (4) of EDTA bleaching of uranyl acetate stain bound to deoxyribonucleoprotein. Specific staining of DNA was obtained by the osmium ammine stain of Cogliati and Gautier (9). These cytochemical procedures were carried out on Epon sections or ultrathin frozen sections.

**Autoradiography.** Cells were exposed to 50 μCi [5-3H]uridine per ml for 10 min to label newly synthesized RNA in the nucleolar fibrils and perichromatin fibrils and then chased with 100 μg unlabeled uridine per ml alone or with 10 or 50 μM quinacrine for periods of 15 min, 30 min, and 2 hr. Ilford L4 emulsion was applied to ultrathin Epon sections on grids by the loop method (22). After 3 months of exposure, development was carried out by the gold extensively-phenid method (8), and the sections were stained selectively for RNP. Grids were examined in Siemens 1A and 101 electron microscopes.

**RESULTS**

**Cell Survival**

In an experiment designed to determine the cytotoxic level of quinacrine under our experimental conditions, culture flasks were seeded with 2.25 × 10⁶ cells. Untreated control hepatocytes examined after being cultured for 3, 24, and 48 hr contained, respectively, 2.15 × 10⁶, 1.35 × 10⁶, and 1.28 × 10⁶ attached cells which excluded trypan blue. When 3- and 24-hr cultures were treated for 30 min with 10 μM quinacrine and then incubated an additional 24 hr in drug-free medium, they contained an average of 1.24 × 10⁶ and 1.32 × 10⁶ viable attached cells, respectively. None of the attached cells were stained with trypsin. Floating cells in both control and treated cultures were all stained. When 3- and 24-hr cultures were treated for 30 min with 50 μM quinacrine, 24 hr later, all cells were detached and stained. In subsequent experiments, therefore, we used total cell detachment as the criterion for cytotoxicity.

**RNA Synthesis**

Quinacrine inhibits the net accumulation of labeled uridine into RNA, the acid-insoluble component, in 24-hr hepatocyte cultures. In the study shown in Table 1, 50 or 100 μCi of [5-3H]uridine per ml were added 5 min after the addition of 10 or 50 μM quinacrine, and the cells were incubated for 15 or 45 min.

The question of whether the reduction in uridine incorporation into RNA by quinacrine might be due to a drop in uridine uptake by hepatocytes was tested. In the studies shown in Table 2, the uptake of labeled uridine into the cells, as well as its incorporation into RNA, was examined at short intervals after administration of 10 μCi [5-3H]uridine per ml medium. The addition of quinacrine inhibited accumulation of uridine in the TCA precipitate (RNA) to a much greater extent than it did accumulation in TCA-soluble pools. The higher dose of quinacrine markedly reduced labeled RNA at 2 and 5 min but did not affect total uptake at these time periods. These data suggest that the primary effect of quinacrine is on the synthesis of RNA rather than the uptake of uridine by hepatocytes.

A series of experiments was also performed in which hepatocytes were first incubated with labeled uridine for 5 min or 2 hr to circumsent effects of quinacrine on uridine uptake. The hepatocytes were washed and incubated for 1 hr with unlabeled uridine with or without quinacrine. In hepatocytes prelabeled for 5 min to label the intracellular uridine pool and subsequently chased 1 hr with unlabeled uridine plus 10 or 50 μM quinacrine, the net accumulation of radioactive uridine in RNA during the 1-hr chase was reduced by quinacrine (Table 3). The net loss of radioactivity over the 1-hr chase suggests that quinacrine might be stimulating RNA degradation. In hepatocytes prelabeled for 2 hr instead of 5 min (Table 3), there was also an inhibition of RNA synthesis and an increased degradation of labeled RNA during a 1-hr incubation with 50 μM quinacrine.

**Nuclear Ultrastructure**

Comparison of the structural changes induced in the nucleus by different doses of quinacrine and exposure times were carried out in hepatocytes which had been cultured for 24 hr before treatment.

**Controls.** Untreated hepatocytes in vitro have condensed chromatin in variable amounts around the nucleolus (perinucleolar chromatin) and in a narrow band contiguous to the inner membrane of the nuclear envelope (perinuclear chromatin). Randomly dispersed small masses in the intervening space appear to represent extensions of the perinuclear and perinuclear chromatin.

### Table 1

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td>Control without quinacrine</td>
<td>8,200</td>
</tr>
<tr>
<td>10 μM quinacrine</td>
<td>10,500</td>
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<tr>
<td>50 μM quinacrine</td>
<td>3,150</td>
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Incorporation of [5-3H]uridine in RNA in the presence of quinacrine

[5-3H]Uridine at concentrations of 50 μCi/ml medium in Experiment 1 and 100 μCi/ml in Experiment 2 was added to 24-hr hepatocyte cultures. Its incorporation into the TCA-precipitable cell component was measured after 15 and 45 min. Quinacrine at doses of 10 or 50 μM was added to test cultures 5 min before addition of the labeled uridine. Each value is the average of 2 counts each of duplicate specimens in each experiment.
nucleolar chromatin. The irregularly oval nucleoli are large loosely organized networks of RNP-containing fibrils and granules. Bleaching of DNA-bound stain to accentuate RNP constituents also reveals fibrils of about 30-Å diameter at the nucleoplasmic border of condensed chromatin (perichromatin fibrils) and in the nucleoplasm (interchromatin fibrils). This stain also reveals a few large 400- to 500-Å perichromatin granules and small clusters of 200- to 250-Å interchromatin granules. Fig. 1 illustrates all of these features as they appear with the preparative procedures used in this study. Current concepts of the functional roles of these structures are discussed in a recent review (32).

Quinacrine-treated Cells. Perinucleolar chromatin appears unchanged at all observed intervals (15 min to 2 hr) of treatment with the nontoxic 10 μM dose of quinacrine. With all toxic doses, it retains its condensed state but becomes pulled away from the inner nuclear membrane (30 min in 50 μM, 15 min in 100 μM, and 5 min in 500 μM), remaining attached to it only by short bridges (Fig. 5) which contain DNA fibrils (Fig. 15). On its opposite surface, an irregular loose network of newly condensed chromatin appears in the adjacent nucleoplasm after 15 min in 50 μM (Fig. 3). During the subsequent 2 hr the chromatin gradually becomes further condensed onto the surface of preexisting heterochromatin (Fig. 4). At higher doses of quinacrine, this condensation of the normally dispersed nucleoplasmic chromatin is more rapid and extensive (Figs. 8 and 9). Whereas 10 μM-treated nuclei appear to be returning to normal after 2 hr, with higher doses (after 6 hr in 50 μM, 2 to 4 hr in 100 μM, and 30 min in 500 μM), all nuclear chromatin becomes dispersed throughout the nucleus into small masses (Fig. 13) interconnected by DNA fibrils (Fig. 15) to form a continuous nodular network.

Perinucleolar chromatin is affected differently. After 30 to 60 min of exposure to 10 μM quinacrine, it is unusually compact and more abundant than usual (Fig. 2). Simultaneously, intranucleolar strands of chromatin (Fig. 1C) retract toward the perinucleolar chromatin. With higher doses (15 min at 50 or 100 μM, and 5 min at 500 μM), the normally condensed chromatin appears to undergo partial decondensation into a network formed by small dense clumps linked together by short strands of linearly oriented fibrils. Sometimes, especially in 500 μM quinacrine, instead of clumps, there are regions of parallel dense bands alternating with extended fibrils, an organization resembling minibands (Fig. 7). Subsequently, perinucleolar chromatin cannot be distinguished from perinuclear chromatin as they join to form the nodular network throughout the nucleus.

The nucleolus exhibits different reactions to nontoxic and toxic doses of quinacrine. It appears normal after 15 min at 10 μM, but after 30 min, it is a small compact sphere, still consisting of intermingled fibrils and granules. After 1 hr, it is almost entirely granular with only traces of fibrils (Fig. 2). After 2 hr, it is returning to normal. On the other hand, with all higher doses, the nucleolus is rapidly fragmented and widely dispersed (Fig. 3), and it becomes entirely fibrillar (Fig. 6). At 50 μM, it later (1 to 2 hr) becomes a roughly spherical more compact mass of fibrils (Fig. 4), but at higher doses, it remains fragmented.

The amount of stainable interchromatin fibrils is usually diminished after 15 min of exposure to 10 μM quinacrine and always reduced after 15 min at 50 or 100 μM and 5 min at 500 μM. It decreases further with longer exposure to the 3 toxic doses, but traces of the precursor perichromatin fibrils usually remain at the borders of the condensed chromatin (Fig. 14). With the nontoxic 10 μM dose, interchromatin fibrils appear to be restored in some cells after 2 hr.

The clusters of interchromatin granules are unaltered by 10 μM quinacrine. At 50 μM, the clusters become larger and more prominent within 15 min. After 30 min, there occur on their periphery one or more small dense nodules (Fig. 12A), amorphous or finely fibrillar. By 1 to 2 hr, these clusters are abnormally large but retain their usual clear or faintly fibrillar background. The clusters are similarly enlarged after 15 min at 100 μM and 5 min at 500 μM. In addition, after 30 min at 100 μM...
and 5 min at 500 μM (Fig. 12), a dense amorphous or finely fibrillar matrix appears among and around the interchromatin granules, except at the center of the cluster (Fig. 13), as though the material were emanating from the peripheral dense nodules. This matrix is not stained by the procedure specific for DNA but is intensely stained by that which binds preferentially to RNP (Fig. 11).

In the cytoplasm, a characteristic of all quinacrine-treated cells is the presence of abundant dense phagosomes. Other cytoplasmic organelles remain normal throughout 2 hr of exposure to 10 μM quinacrine. At doses of 50 and 100 μM, nonspecific cytotoxic changes first appear after 6 and 4 hr, respectively, when nuclear chromatin assumes its "nodular network" form. The latter nuclear changes already are evident after 30 min in 500 μM, before cytoplasmic changes have occurred.

**Autoradiography**

In control preparations, 10 min of exposure to 50 μCi [5-3H]uridine per ml labels the fibrillar component of the nucleolus but not the granules, and it labels the nucleoplasmic borders of the perinuclear and perinucleolar chromatin, the areas occupied by perichromatin fibrils. A chase with 100 μg unlabeled uridine per ml for 15 min induces no change, but after chases of 30 min and 2 hr (Fig. 10), label occurs over the granular component of the nucleolus and over the interchromatin fibrils. Preparations chased for the same periods with unlabeled uridine plus 10 μM quinacrine exhibit distributions of autoradiographic silver grains identical with those of the controls. When 100 μM quinacrine is added to the chase medium, the same structures are labeled, and in addition, silver grains are abundant around the periphery of the large clusters of interchromatin granules (Fig. 11). The labeled nucleoli after 30-min and 2-hr chases with 100 μM quinacrine are entirely fibrillar and fragmented.

**DISCUSSION**

In untreated control hepatocyte cultures, there is an initial drop in the average number of viable cells during the first 24 hr of incubation but no loss between 24 and 48 hr. We arbitrarily selected a 30-min exposure of the cells to quinacrine to determine cytotoxic levels of the drug. When either 3- or 24-hr cultures are treated for 30 min with 10 μM quinacrine, 24 hr later, the number of surviving cells is similar to that in corresponding 24- or 48-hr control cultures, which indicated that this dose is nontoxic. Doses of 50 to 500 μM for 30 min, on the other hand, do not permit any subsequent recovery and survival for 24 hr. These higher doses of quinacrine are useful, however, to accentuate certain short-term effects of the drug.

Our observation that quinacrine reduces the net accumulation of labeled uridine in the TCA-precipitable RNA of isolated hepatocytes in vitro corroborates results obtained with a variety of other biological models (28). The mechanisms involved, however, remain to be elucidated. We do not believe that the primary effect is on the uptake of uridine into the cell pool, because 50 μM quinacrine reduces uridine incorporation in RNA before it exhibits any effect on total uptake, and at later intervals, when uptake into the soluble pools is partially inhibited, the inhibition of uridine accumulation in RNA is much greater. We suggest, instead, that the major effect of quinacrine is on RNA synthesis and degradation in the nucleus.

Quinacrine at all doses used in this study induces both a loss of RNP interchromatin fibrils and an alteration of nucleolar structure. Bachellerie et al. (1), in a combined biochemical and autoradiographic study, demonstrated that perichromatin fibrils are the first extranucleolar structures to be labeled with tritiated uridine and that they contain heterogeneous RNP. In a later quantitative autoradiographic investigation, Puvion and Moyne (31) showed that these fibrils then migrate from their site of synthesis at the borders of condensed chromatin to become the interchromatin fibrils. On this basis, we can tentatively conclude that at least a part of the reduction in RNA synthesis brought about by quinacrine may represent increased degradation of heterogeneous RNA. In our material treated with 10 μM quinacrine, some nuclei reveal diminished RNP staining of interchromatin fibrils before nucleolar alterations are visible. This supports the view (27) that a product of heterogeneous RNA may control the synthesis of rRNA.

Studies of nucleolar incorporation of labeled uridine into preribosomal RNA demonstrated that synthesis occurs at the site of the nucleolar fibrils, and during subsequent processing of the rRNA, the label migrates into the nucleolar granules (19). Our observed loss of nucleolar fibrils in the presence of 10 μM quinacrine suggests that reduction of rRNA synthesis occurs. The persistence of nucleolar granules with this dose of the drug and our autoradiographic demonstration of labeled uridine in them suggest that the processing of existing fibrils into granules continues.

On the other hand, with the higher dose of 50 μM quinacrine, nucleolar constituents are primarily fibrillar with only a few granules persisting. A similar effect of quinacrine (15 μg/ml) on nucleoli has been described in L-strain mouse fibroblasts (20). This is morphological evidence for disruption of normal rRNA turnover, and it is supported by our incorporation studies. With doses of 100 or 500 μM, the nucleoli are already entirely fibrillar at the earliest time points examined. This persistence of nucleolar fibrils might represent a more rapid synthesis of fibrils, but this is not supported by our incorporation studies. Another possible interpretation is that the RNA-containing granules unwind to again become fibrils, a phenomenon described in hyperthermic shock (34) which blocks rRNA processing. This might be possible in our material, since prelabeled nuclei chased 2 hr in the presence of 100 μM quinacrine contain nucleoli which are both entirely fibrillar and heavily labeled. On the other hand, recent reports (14, 24) have shown that another compound, D-galactosamine, completely blocks RNA synthesis without blocking its processing at a time when the nucleolus fragments and consists entirely of fibrils. These fibrillar remnants of nucleoli are devoid of RNA (24) whereas ours are labeled with tritiated uridine. In autoradiographs of [3H]uridine-treated control cells, the grains do represent sites of RNA since they can be destroyed by RNase digestion (17). However, we have not yet ruled out the possibility that in our quinacrine-treated cells the autoradiographic grains may represent only persisting fragments of previously labeled RNA. Therefore, at this time, we cannot ascribe a specific metabolic change to this type of nucleolar abnormality. It seems necessary, instead, to attempt to interpret nucleolar changes in association with the ensemble of alterations in the surrounding chromatin.
Other intercalating agents (5) induce nucleolar segregation and enspherulation. Quinacrine rarely produces segregation. However, at the 10 μM concentration but not higher doses, it does produce nucleolar enspherulation. As with other intercalating agents, this enspherulation would appear to be the result of the contraction of intranucleolar DNA.

Chromatin structure in interphase nuclei undergoes dramatic changes following intercalation of quinacrine into the DNA. Our low dose (10 μM) visibly affects nucleolar chromatin, causing a seemingly increased compactness of perinuclear chromatin, which reduces the surface area available for perichromatin fibril formation, and also produces a retraction of the intranucleolar fibrils, which presumably reduces the amount of DNA available for preribosomal RNA transcription. This appears to be a differential effect because other types of chromatin exhibit no change detectable with our methods at this dose of quinacrine. The toxic doses, on the other hand, alter 3 types of chromatin, the extent varying with the dose. There appears to be a partial decondensation of perinucleolar chromatin to form short segments of extended fibrils alternating with dense clumps or bands. The latter configuration has been seen in nucleolar chromatin of Chironomus salivary glands after exposure to actinomycin D (36). Conversely, the rest of the nuclear chromatin clearly undergoes condensation. The normally dispersed and invisible nucleoplasmic chromatin (13) condenses very rapidly to become visible as a widespread loose network which then retracts toward the periphery of the nucleus. To our knowledge, this effect has not been described previously. Puvion and Moyne (31) demonstrated a coincidence in timing of the migration of extranucleolar RNP fibrils from their site of synthesis along the boundaries of condensed chromatin (perichromatin fibrils) into the nucleoplasm or interchromatin space (interchromatin fibrils) and the decondensation and dispersion into the nucleoplasm of previously condensed perinuclear chromatin. They suggested that there may be a physical association between the RNP fibrils and chromatin fibrils, the latter acting as carriers for the former. In this work, the nucleoplasmic chromatin undergoes the opposite reorganization, from a dispersed to a peripherally contracted form. This might account for some of the diminution in detectable interchromatin fibrils and for some persistence of perinucleolar granules which are storage forms of perichromatin fibrils. Finally, some further condensation of perinuclear chromatin seems to occur since it pulls away or retracts from the nuclear envelope to the extent allowable by its points of attachment to the inner nuclear membrane. This phenomenon has been described previously after exposure of cells to several compounds which intercalate into DNA (35) and recently has been detected after treatment of synovial cells with gold sodium thiomalate (26). It is difficult to reconcile a decondensation of perinuclear chromatin with condensation of perinuclear and nucleoplasmic chromatin if one adopts the attractive concept (3, 21) that all nuclear chromatin forms a continuous system. It is possible, however, that condensation does occur throughout the system and is expressed in the perinuclear zone by the small masses and parallel bands but that strains caused by the sudden contraction produces regions of stretched fibrils in perinucleolar chromatin which would be analogous to the fibrillar bridges which bind retracted perinuclear chromatin to the nuclear envelope. On the other hand, whole mount electron microscopy of actinomycin D-banded chromosomes (11) demonstrated that differential condensation can occur. In either case, perinucleolar chromatin differs from other interphase chromatin in its response to quinacrine.

Interchromatin granules normally occur in small clusters randomly situated in the nucleoplasm. Sometimes they appear to be connected by thin fibrils (37). After treatment of hepatocytes with 100 or 500 μM quinacrine, the size of the clusters increases rapidly, one or more dense nodules appear at their periphery, and a wide peripheral band of deeply staining amorphous matrix appears among and around the granules. We cannot tell at this time whether the larger clusters represent an increase in number of the granules or the merging of smaller clusters. To our knowledge, the dense matrix in which the interchromatin granules become embedded has not been described previously. This matrix is not destained by EDTA and therefore may represent an hitherto unseen type of RNP. Although the granules also are not destained by EDTA, it has been suggested that they consist of phosphorylated proteins instead of RNP (37). In cells prelabeled 10 min with [5-3H]uridine and chased 2 hr in the presence of 100 μM quinacrine, some of the label in autoradiographs is concentrated around the periphery but not within the enlarged clusters of interchromatin granules. This corroborates an earlier study (18) and supports the concept that the granules and possibly their associated dense matrix may not contain RNP. The nature and role of interchromatin granules remain unclear, as does the significance of the increase in cluster size and formation of an associated matrix material. Inasmuch as the interchromatin granules are considered to be components of the "nuclear matrix" (3), their alteration in the presence of high doses of quinacrine may represent underlying changes in the structure of a dynamic protein framework in the nucleus.

REFERENCES


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