

THE DISORGANIZATION OF HEPATIC CELL NUCLEOLI INDUCED BY ETHIONINE AND ITS REVERSAL BY ADENINE

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ABSTRACT

The structure of nuclei and nucleoli of hepatic cells after short-term ethionine administration was investigated with the electron microscope. By 1½ hr after the injection, a distinct alteration occurred in the nucleoli which was characterized by the appearance of electron-opaque masses in the nucleolonema. After 6–8 hr, the nucleoli showed partial fragmentation into small, dense masses. Large aggregates of interchromatinic granules appeared in the nucleoplasm. Condensation of chromatin became prominent in the nucleoplasm particularly along the nuclear membrane. By 12 hr almost complete fragmentation of nucleoli had occurred. The administration of adenine or methionine at 4 hr prevented the development of nucleolar changes. Also, adenine administration at 8 hr after ethionine completely reversed the nucleolar lesion by 12 hr. After methionine administration at 8 hr, many nucleoli showed incomplete reconstruction with many twisted ropelike structures when viewed 4 hr later. Identical structures were found when adenine was given at 8 hr, and animals were sacrificed 2 hr later. On the basis of this observation, the simplified structures of nucleoli found 2 hr after adenine or 4 hr after methionine appeared to be precursors of the nucleolonema. It is suggested that nucleoli show at least two basic reaction patterns to inhibitors of RNA synthesis, one typified by actinomycin D and one by ethionine.

INTRODUCTION

The administration of ethionine to female rats is followed by a sequence of biochemical and morphologic alterations in the liver which appear to be initiated by a rapid fall in the adenosine triphosphate (ATP) concentration (1–4). The decrease in ATP concentration is maximal within 2 hr and is the result of an imbalance between the rate of trapping of adenosine as *S*-adenosylethionine and the rate of *de novo* synthesis of adenine nucleotides (5). The hepatic ATP deficiency is, in turn, followed by a striking inhibition of RNA (6, 7) and protein synthesis (3, 8). The alteration

in protein synthesis is accompanied by changes in the function and ultrastructure of liver polysomes (9, 10). The ATP deficiency and its consequences are readily reversed by the administration of several ATP precursors such as adenine, inosine, or 5-amino imidazole-4-carboxamide (3, 5, 8, 11).

Further observations of the ultrastructural consequences of the short-term metabolic effects of ethionine have revealed striking and highly reproducible alterations in the nuclei and nucleoli of hepatic cells. The nucleolus undergoes a progressive disorganization with eventual fragmenta-

tion and scattering of its normal components. Changes in the chromatin distribution and nucleoplasm also occur. These structural alterations in the nucleus and nucleolus are remarkably reversible when the basic ATP deficiency is counteracted by the administration of ATP precursors such as adenine or the natural metabolite of ethionine, methionine. The organizational pattern of the reformation of the nucleolus is so highly reproducible as to suggest that this model may be of considerable interest and value in the analysis of the structure of the liver cell nucleolus in relation to its function. The detailed description of the nucleolar alterations and their apparent return to normal are the subject of this paper.

MATERIALS AND METHODS

White female rats of the Wistar strain (Carworth Farm, New City, N. Y.) maintained on Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.) and weighing 175–200 g were deprived of food overnight before the

start of each experiment. The experiments were begun between 7 and 8 a.m. by the intraperitoneal injection of DL-ethionine (1 mg/g body weight) in an aqueous solution containing 25 mg/ml. Methionine was given in an aqueous solution in an amount equimolar to ethionine, while adenine was administered as adenine sulfate in an aqueous solution in a dose of 0.16 mmole (32 mg). Both compounds were given intraperitoneally. Control animals received intraperitoneal injections of distilled water or saline in volumes equal to those administered to the experimental animals. The times of sacrifice and the exact sequence of the administration of adenine or methionine are given in Table I.

Tissue was removed from the left lateral lobe of the liver while the animal was under ether anesthesia or immediately after decapitation. Small pieces of tissue were fixed in 1% osmium tetroxide in phosphate buffer (pH 7.3) or 2% osmium tetroxide in *s*-collidine buffer (pH 7.4). The tissues were dehydrated at room temperature in a graded series of ethanol solutions and were embedded in Epon 812 by the Luft (12) method. Sections were cut on Porter-Blum MT2

TABLE I
Experimental Schema

Initial treatment	Secondary treatment	Interval between first and second treatment	Time of sacrifice after the initial treatment*
		hr	hr
Ethionine	—	—	½ (2), 1 (3), 1½ (2), 2 (3)
Ethionine	—	—	4 (6), 6 (7), 8 (4), 12 (4)
Ethionine	Adenine and methionine	4	8 (2)
Ethionine	Adenine	4	8 (3)
Ethionine	Methionine	4	8 (2)
Ethionine	Saline	4	8 (3)
Ethionine	Adenine and methionine	8	12 (2)
Ethionine	Adenine	8	12 (3)
Ethionine	Adenine	8	10 (2)
Ethionine	Methionine	8	12 (4)
Ethionine	Saline	8	12 (3)
Control:			
Distilled water	---	—	½ (1), 1 (1), 1½ (1), 2 (1), 4 (2), 6 (2), 8 (2), 12 (2)
normal saline	---	—	
Adenine	---	—	4 (2)
Methionine	---	—	4 (2)

* The number of animals is in parenthesis.

ultramicrotomes with glass knives and then were picked up on uncoated grids and stained with lead hydroxide by the Karnovsky (13) method. The sections were examined in a Philips 100 B electron microscope with a 60 kv acceleration voltage.

RESULTS

Control Rats

The fine structure of normal rat liver nuclei has been described in detail (14, 15). Since our observations on osmium tetroxide-fixed material

do not differ significantly from those in previous studies, we have adopted the same terminology for the various components of the nuclear structure with the modifications recently agreed upon at the International Symposium on the nucleolus (16).

Rats given an injection of distilled water or saline and sacrificed at various intervals showed no abnormalities in nuclear or nucleolar structure. The nucleus presented smooth, round, or ovoid contours with a distinct nuclear membrane (Fig. 1).

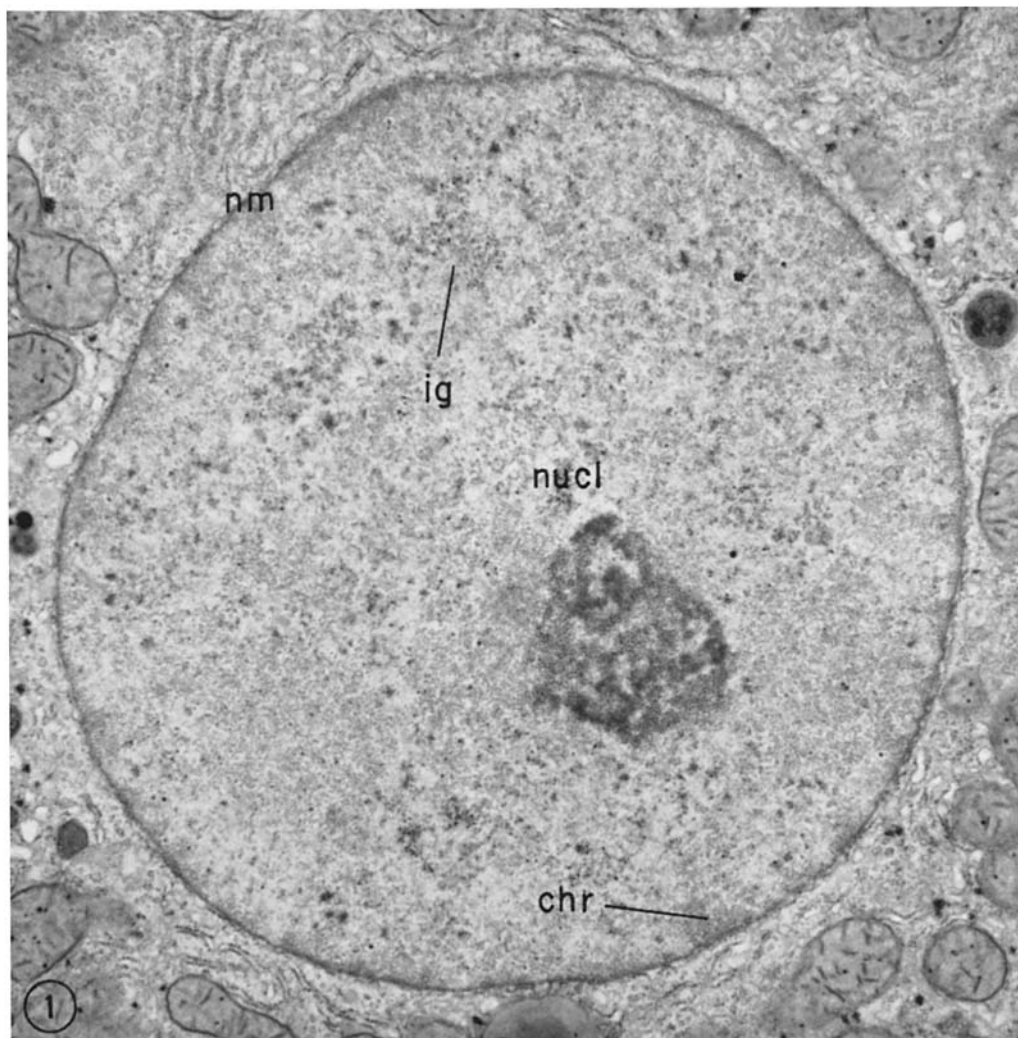


FIGURE 1 Hepatic cell nucleus of control rat. Nucleus is roughly round and shows a distinct nuclear membrane (*nm*). Nucleolus (*nucl*) is compact with a smooth contour. Chromatin (*chr*) is indistinct, and is only faintly visible along the nuclear membrane. A small cluster of interchromatinic granules (*ig*) is seen in the left upper part of the nucleus. $\times 14400$.

The interchromatinic substance was evenly distributed and had a low electron opacity. The chromatin pattern was rather indistinct in material fixed with osmium tetroxide buffered with either phosphate or *s*-collidine and could be seen only faintly along the nuclear membrane and around the nucleolus. Interchromatinic granules occasionally could be seen appearing in small poorly circumscribed clusters (Fig. 1). Perichromatin granules were also seen infrequently. Hepatic parenchymal cells contained one to several nucleoli, although nuclei with only nucleolar fragments or no nucleoli were frequently encountered in thin sections. Nucleoli appeared centrally placed or disposed close to the nuclear membrane. They were compact, roughly oval or round, and composed of at least two elements: dense granules 100–150 Å in diameter (particulate component), arranged in clumps or loose aggregates, and a somewhat less dense structure made up of 80–100-Å fibrils (fibrillar component) (Fig. 2). These elements were arranged in a ropelike or skeinlike pattern (nucleolonema) enveloping small irregular areas called nucleolar vacuoles (*pars amorpha*).

30 Min–4 Hr After Ethionine Injection

In rats sacrificed 30 min or 1 hr after the injection of ethionine, no abnormalities of the nucleus or nucleolus were seen. At 1½ hr, nuclei retained their normal contour, and chromatin was dispersed. However, there was an apparent increase in prominence of interchromatinic granules. At this time, the nucleolus exhibited a distinct alteration which involved the majority of nuclei and was seen in all nucleoli within a single cell. The abnormality consisted of aggregates of dense material within the nucleolonema and nucleolar vacuoles or at the periphery of the nucleolus in the region of the nucleolus-associated chromatin (Fig. 4). The aggregates were of variable size and appeared to be composed of a dense, fibrillar material on which granular elements were superimposed. The particulate and fibrillar components and nucleolar vacuoles were preserved, and no segregation of components was seen. The changes in the nucleolus at 2 and 4 hr were very much the same. The dense aggregates appeared to have increased in average size though their morphology and distribution were the same as those present at 1½ hr (Figs. 3, 5). At 4 hr, many cells exhibited a very distinct chromatin pattern (Fig. 3). Chromatin aggre-

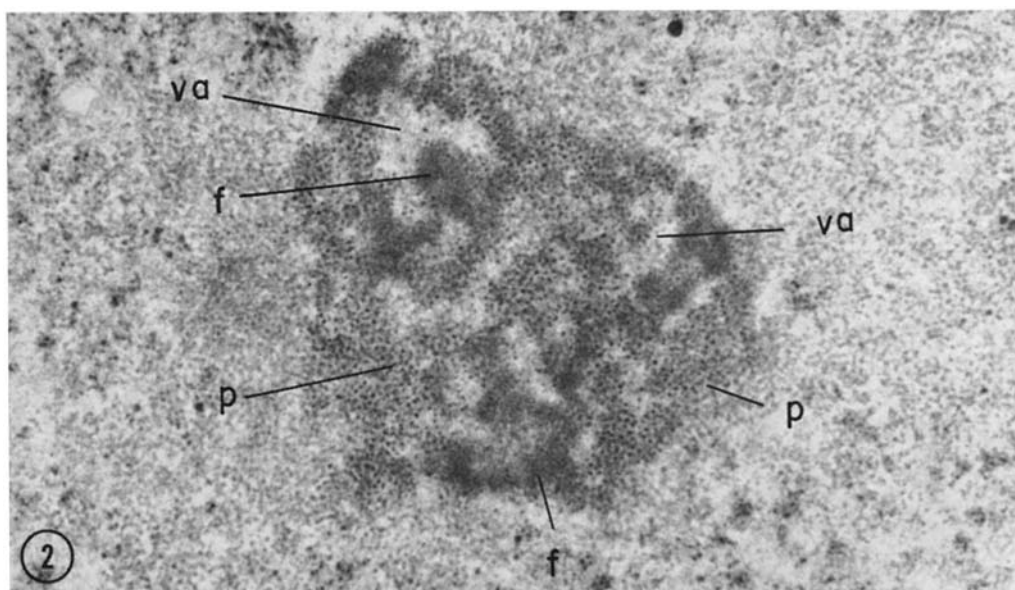


FIGURE 2 High magnification view of nucleolus from Fig. 1. The fibrillar (*f*) and particulate (*p*) components of the nucleolonema are intimately intermingled. The nucleolar vacuoles (*va*) are irregular and show an indefinite outline. $\times 39200$.

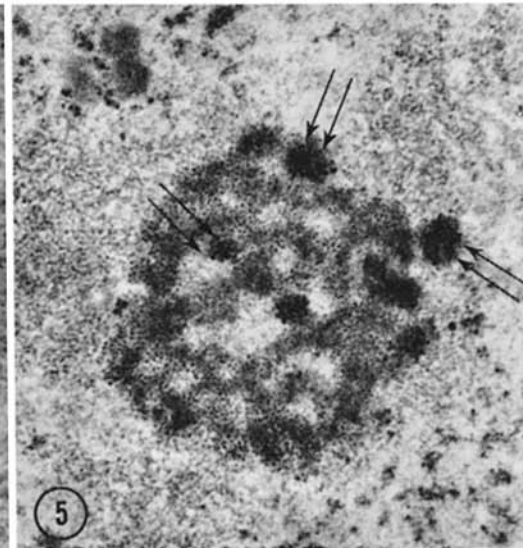
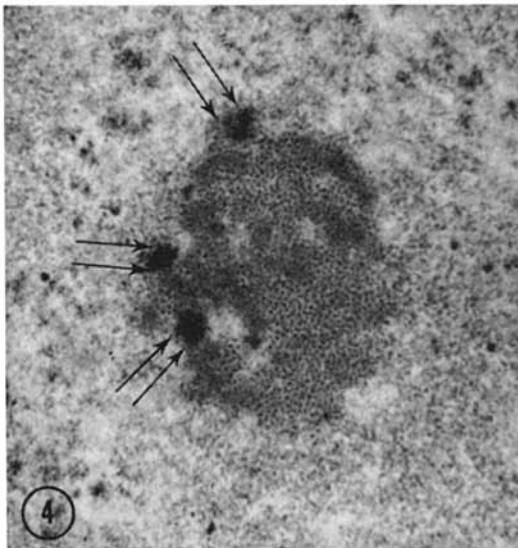
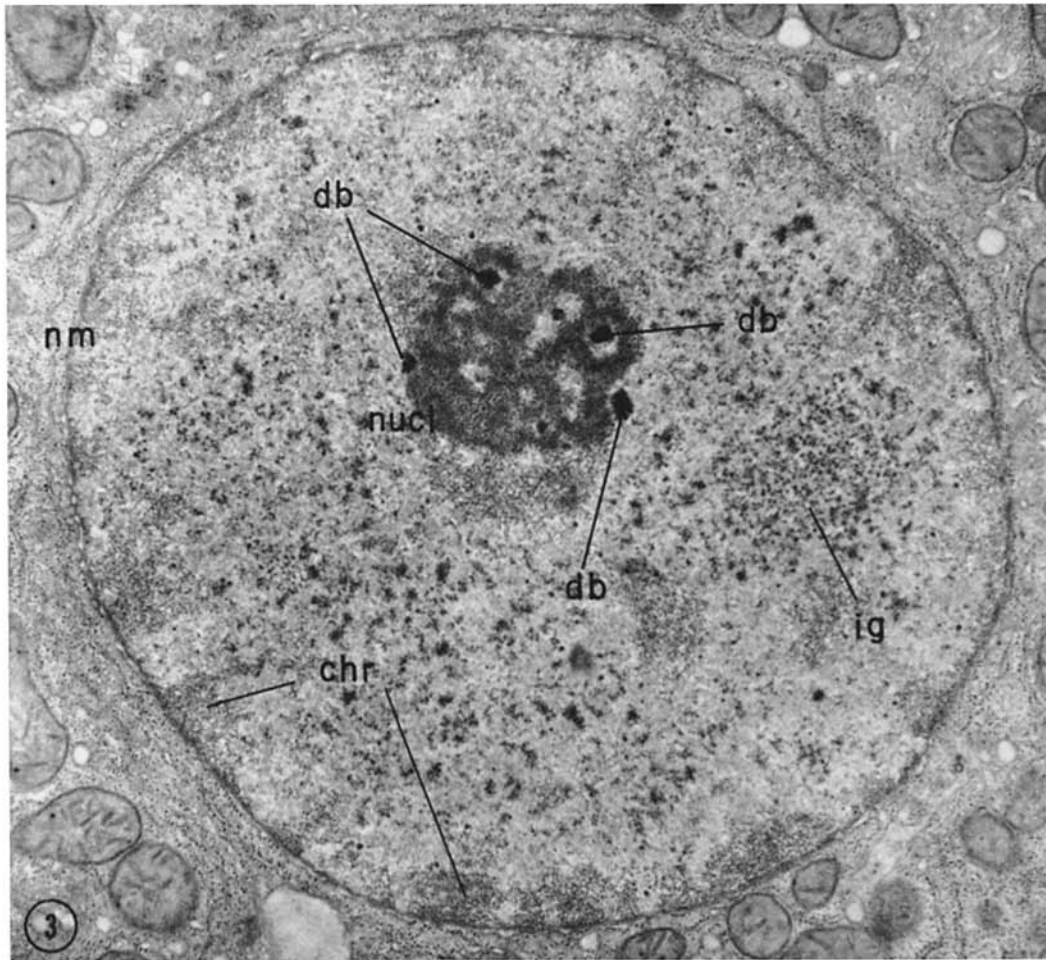


FIGURE 3 Hepatic cell nucleus of rat sacrificed 4 hr after the ethionine injection. Nucleolus (*nucl*) still shows normal contour and compactness. Electron-opaque masses (*db*) appear in the nucleolus. Note the prominence of chromatin (*chr*) pattern along the nuclear membrane (*nm*). Interchromatinic granules (*ig*) are also increased. $\times 15600$.

FIGURE 4 Nucleolus of rat liver cell 2 hr after the ethionine injection. Double arrows indicate electron-opaque masses. Particulate and fibrillar components of nucleolus still remain. $\times 20800$.

FIGURE 5 Another view of the electron-opaque masses (double arrows) in the nucleolus of rat liver cell 4 hr after the ethionine injection. Note the persistence of nucleolonema with nucleolar vacuoles. $\times 25300$.

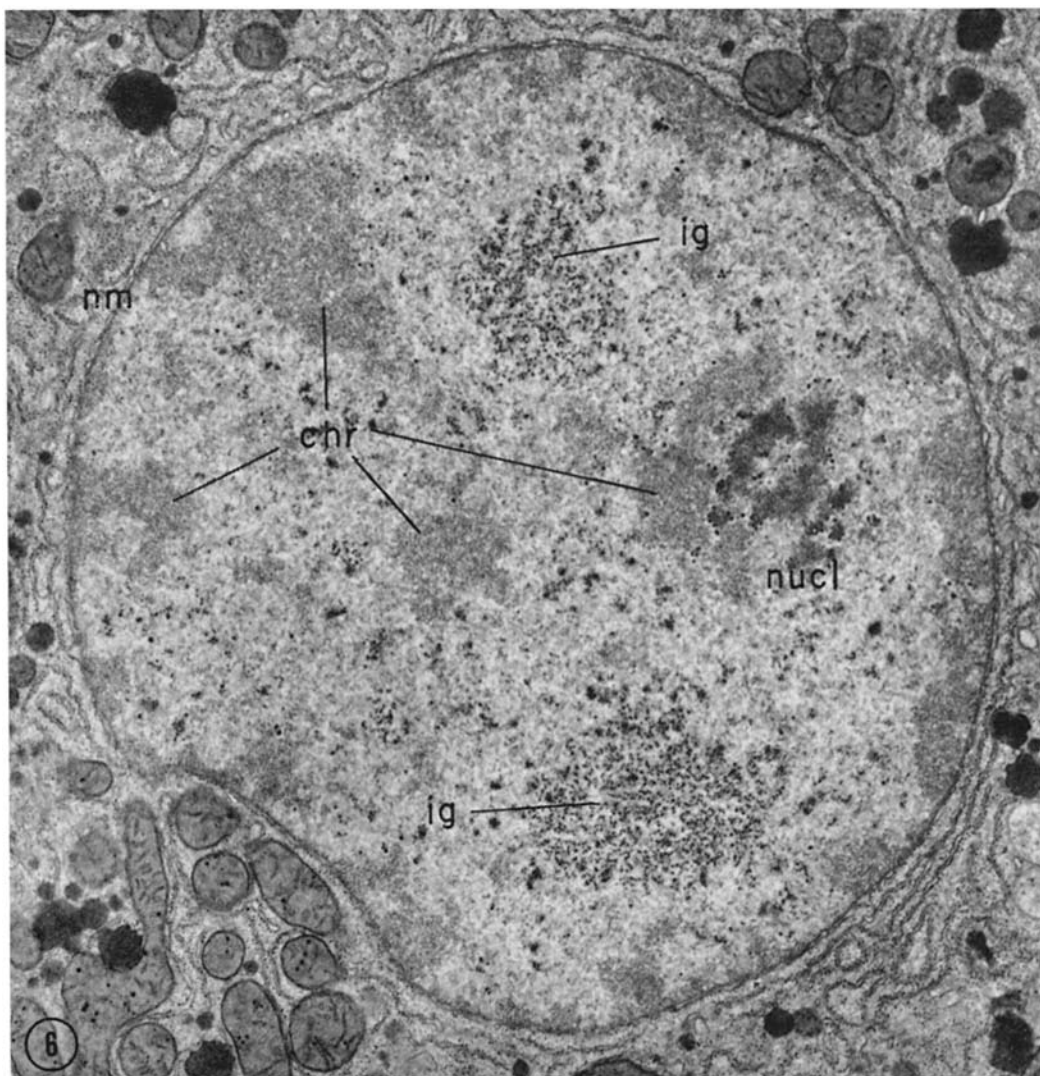


FIGURE 6 Typical nuclear changes in hepatic cell of rat sacrificed 6 hr after the ethionine injection. Chromatin (*chr*) is prominent around the nuclear membrane (*nm*) and periphery of nucleolus (*nucl*). Nucleolus is fragmented. Two distinct areas of aggregates of interchromatinic granules (*ig*) are seen. $\times 13500$.

gates were prominent around the nuclear membrane as well as in the area adjacent to the nucleoli. In contrast to the nucleolar changes, the change in chromatin pattern was not observed in all cells.

6-8 Hr After Ethionine Injection

The normal contour of the nucleolus began to distort. The nucleolus was no longer compact

(Fig. 6), and there were areas which showed separation of fibrillar and particulate components (Fig. 7). In addition, fragmentation of nucleolar components into small spherular electron-opaque masses was evident (Figs. 7-9). These spherules appeared in the vicinity of nucleolar remnants. Scattered among these fragments were many granules measuring up to 300 Å in diameter. Many of these granules were seen in the periphery

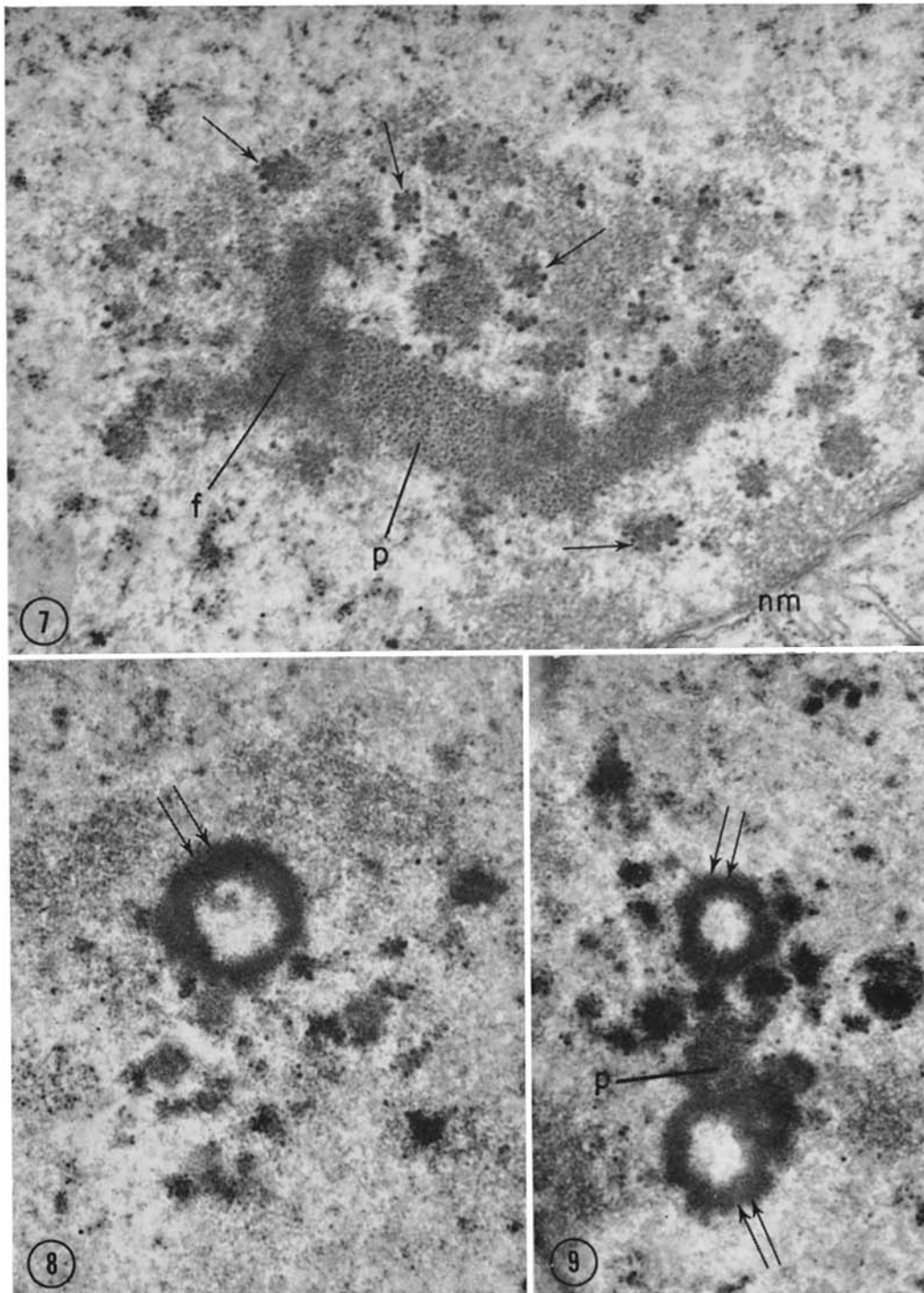
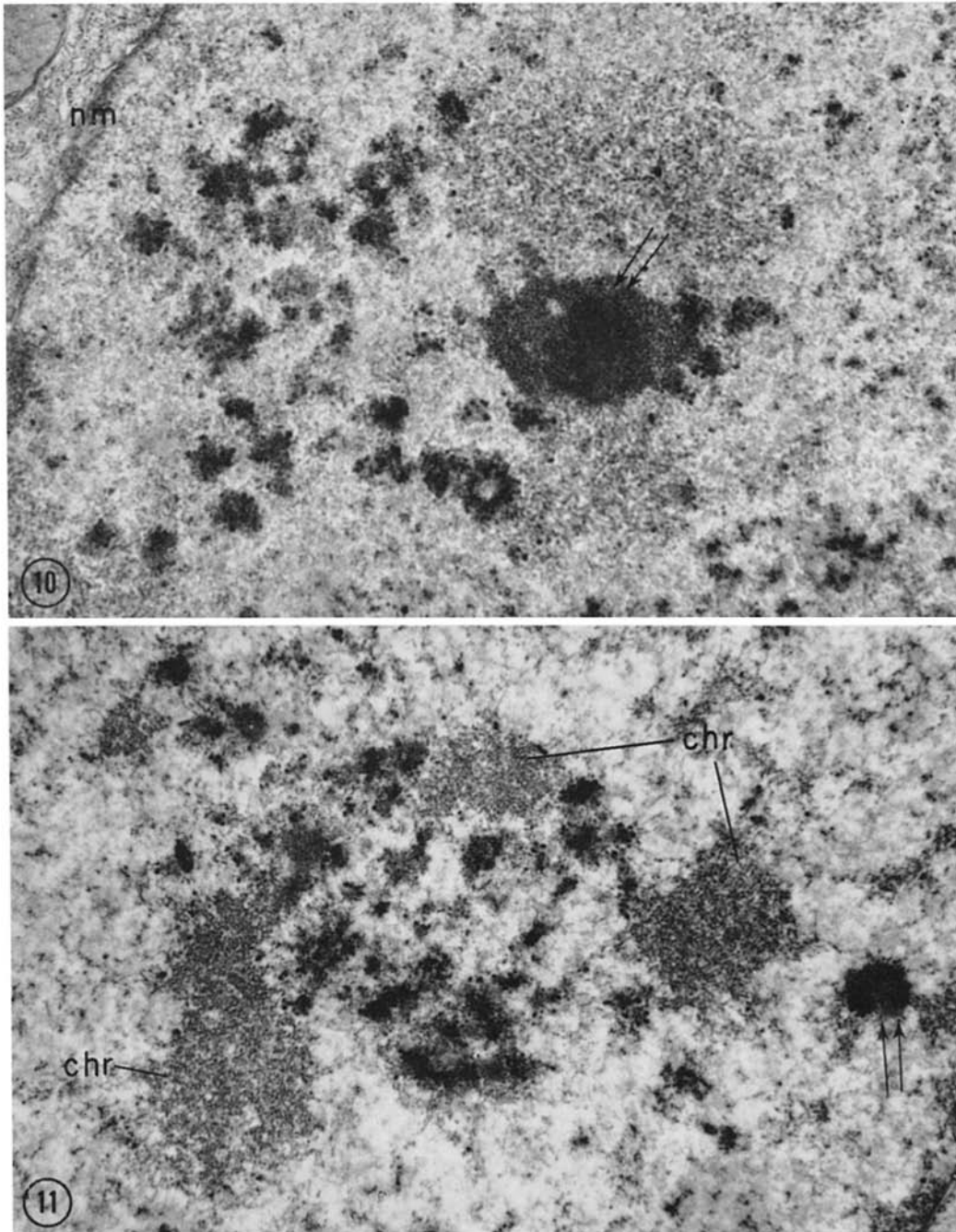


FIGURE 7 High magnification view of disorganized nucleolus of hepatic cell 8 hr after the ethionine injection. Remnant of nucleolus still shows particulate (*p*) and fibrillar (*f*) components. Small fragments are indicated by arrows. Peripheral satellite granules are prominent. *nm*, nuclear membrane. $\times 31500$.

FIGURES 8 and 9 Fragmentation of nucleoli 8 hr after the ethionine injection. Note the ring-shaped nucleolar remnants (double arrows). Fig. 8, $\times 24500$; Fig. 9, $\times 21800$.



FIGURES 10 and 11 Nucleolar fragmentation observed 12 hr after the ethionine injection. Small masses indicated by double arrows probably represent remnants of nucleoli. Chromatin (*chr*) is very distinct in the vicinity of fragments. *nm*, nuclear membrane. Fig. 10, $\times 22000$; Fig. 11, $\times 21000$.

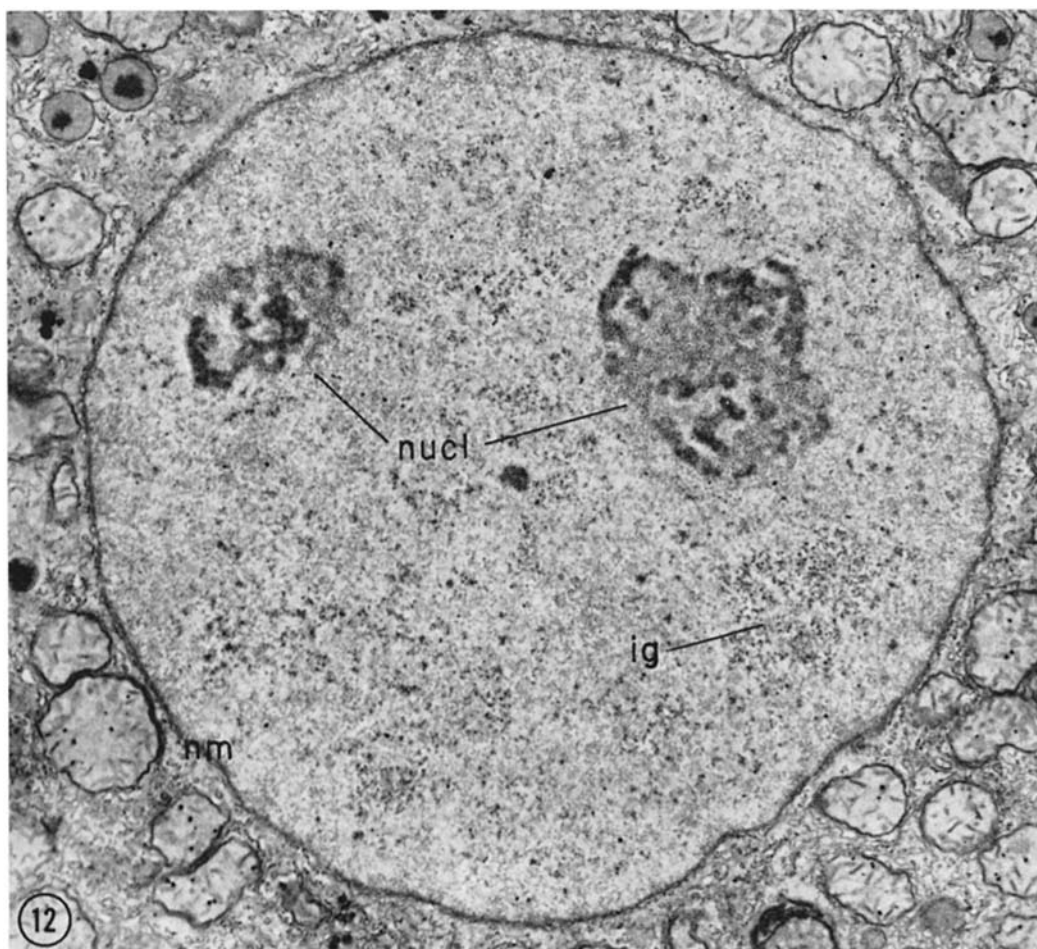


FIGURE 12 Low magnification view of hepatic cell nucleus of rat given mixture of adenine and methionine 8 hr after the ethionine injection and sacrificed 4 hr later. Two fairly compact nucleoli (*nucl*) show formation of nucleolonema. Accentuated chromatin pattern has disappeared (compare Fig. 6). Aggregates of interchromatinic granules (*ig*) still remain. *nm*, nuclear membrane. $\times 13000$.

of individual spherules and formed a satellite appearance (Figs. 7, 8). Again, these nucleolar alterations can be seen in nearly all hepatic parenchymal cells, though there are minor differences in configuration from cell to cell.

Well-circumscribed aggregates of interchromatinic granules appeared in the nucleoplasm. Frequently two or three such areas were seen in a single nucleus (Fig. 6). These aggregates of interchromatinic granules were disposed in irregular clusters. In addition, the distribution of the nuclear chromatin became very distinct at this time. Tissue which was fixed in phosphate-buffered osmium tetroxide and stained with lead hydroxide

exhibited a remarkably strong staining of chromatin, especially around the nuclear membrane and around the nucleolus (Fig. 6). The scattered islands of chromatin in the interchromatinic substance also became distinct.

12 Hr After Ethionine Injection

The nucleolar lesions showed further progression, and there was nearly complete fragmentation of the nucleolus (Figs. 10, 11). Each fragment appeared to be much denser than the normal nucleolar components and was frequently surrounded by satellite granules. Clumps of chromatin could be seen between these fragments. In

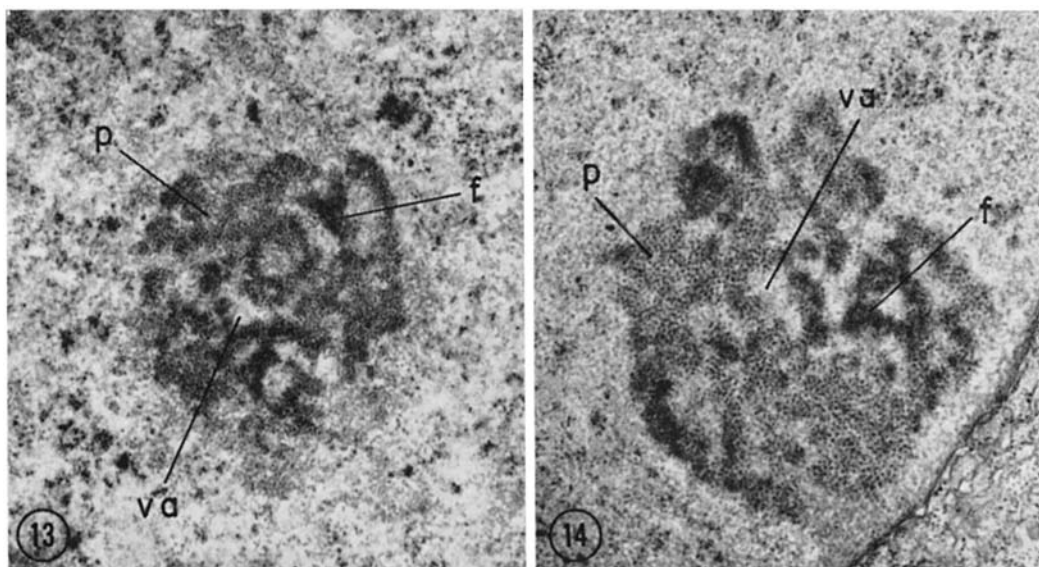


FIGURE 13 Hepatic cell nucleolus of rat given adenine 4 hr after the ethionine injection and sacrificed 4 hr later. Note the disappearance of electron-opaque masses (compare Figs. 3, 5). Nucleolus shows two components, fibrillar (*f*) and particulate (*p*), and nucleolar vacuoles (*va*). $\times 16700$.

FIGURE 14 Hepatic cell nucleolus of rat given adenine 8 hr after the ethionine injection and sacrificed 4 hr later. Nearly complete restoration of nucleolar structure is evident. Particulate (*p*) and fibrillar (*f*) components with nucleolar vacuoles (*va*). $\times 23300$.

the immediate vicinity of these fragments we often observed a small mass that appeared to be a remnant of the nucleolus. These masses sometimes consisted entirely of fibrillar components similar to those of normal nucleoli, but sometimes a combination of fibrillar and particulate components was also seen. The distinct chromatin pattern in the nucleoplasm and the aggregates of interchromatinic granules remained the same as those seen in the lesion in the 6–8 hr period.

Effect of Adenine, Methionine, or Mixture of Both Given 4 Hr After Ethionine

A mixture of adenine and methionine, when given 4 hr after the injection of ethionine, completely abolished the nuclear alterations seen at the 8 hr period. The margination of chromatin along the nuclear membrane completely disappeared. The accentuation of clumps of chromatin in the nucleoplasm also disappeared, as well as the chromatin in the periphery of the nucleolus. The electron-opaque masses, which were present in the nucleolus at the $1\frac{1}{2}$ –4 hr period, were not seen. Fragmentation of nucleoli, so prominent in

the nuclei of the 8 hr period, was no longer present. The nucleoli showed a fairly compact structure with normal contours. At high magnification, normal nucleolar components could be seen. In separate experiments it was found that either adenine or methionine, given 4 hr after the ethionine injection, was as effective as the mixture in protecting against the induction of the nucleolar lesions induced by ethionine (Fig. 13).

Effect of Mixture of Adenine and Methionine or Adenine Alone Given 8 Hr After Ethionine

When rats were given a mixture of adenine and methionine 8 hr after the injection of ethionine and were sacrificed 4 hr later, the nucleolar disorganization seen at the 8–12 hr period was no longer present (Fig. 12). The majority of the nucleoli were normal morphologically and exhibited two distinct components and intermingled nucleolar vacuoles. An occasional nucleolus displayed slightly irregular contours. The accentuation of chromatin pattern and the aggregates of

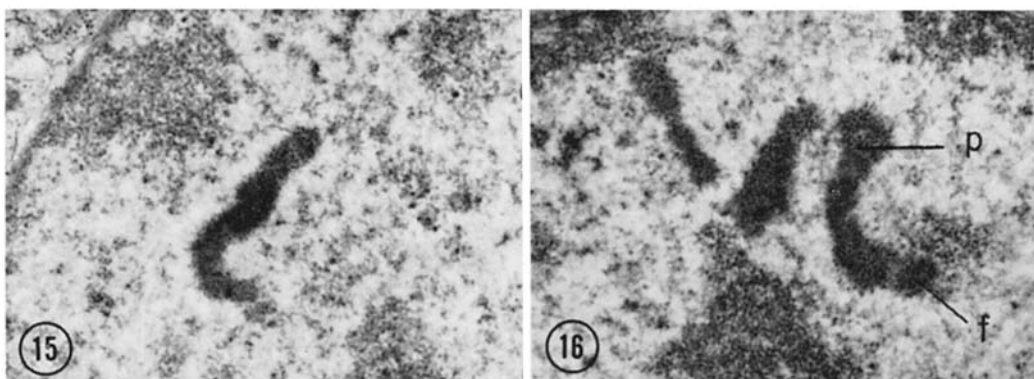


FIGURE 15 A simple rodlike structure found in nucleus of hepatic cell in rat given methionine 8 hr after the ethionine injection and sacrificed 4 hr later. $\times 20500$.

FIGURE 16 Three rodlike structures found in nucleus of hepatic cell in rat given adenine 8 hr after the ethionine injection and sacrificed 2 hr later. The rodlike structure consists of particulate (*p*) and fibrillar (*f*) elements of nucleolus. $\times 18600$.

interchromatinic granules appeared to have diminished (Fig. 12).

Adenine alone was just as effective in inducing the restoration of normal nucleolar organization 4 hr after its administration as the combination of adenine and methionine (Fig. 14). It must be stressed that the restoration of nucleolar structure after the administration of adenine, with or without methionine, can be seen in virtually every hepatic parenchymal cell.

Effect of Methionine Alone Given 8 Hr After Ethionine

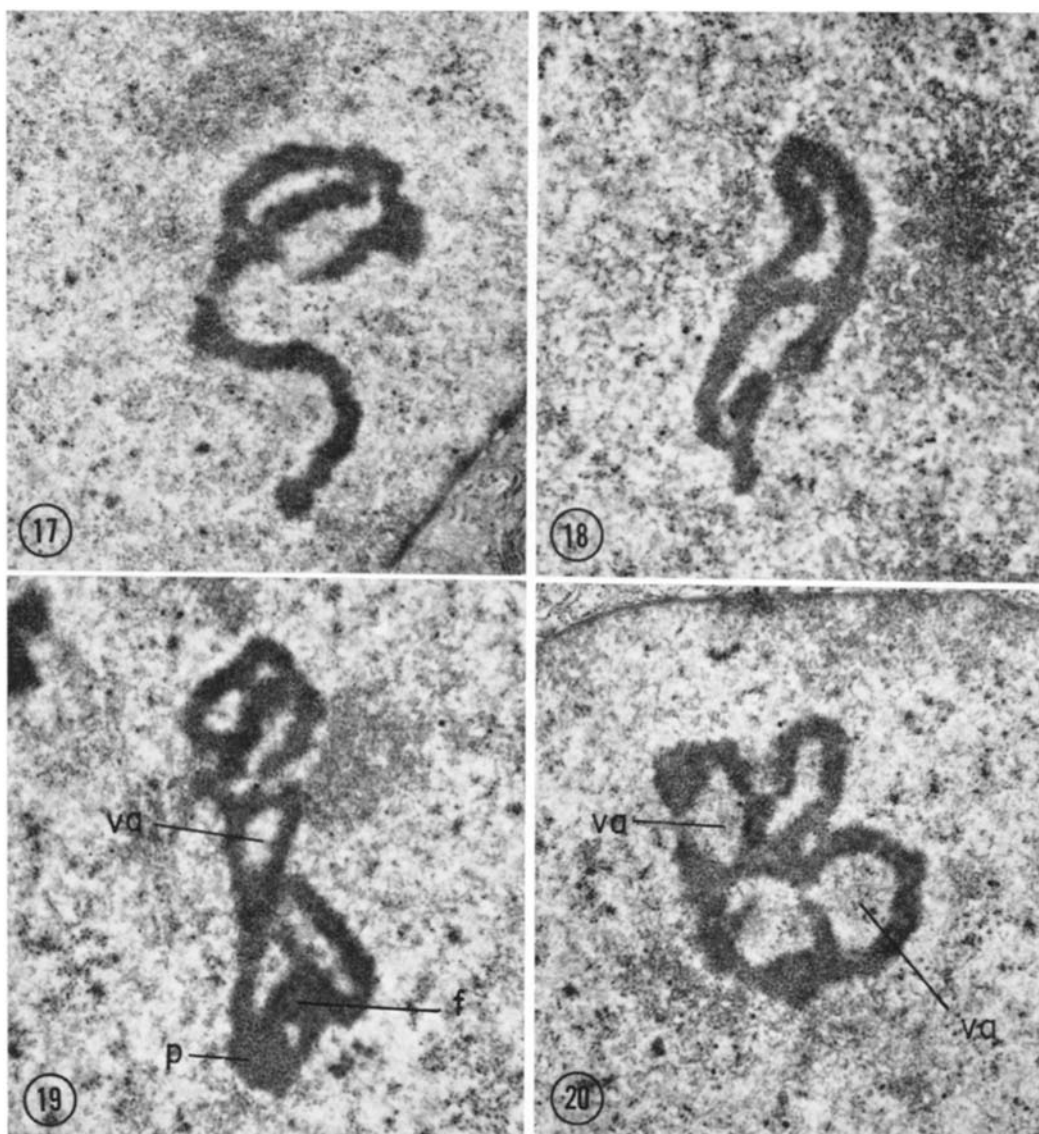
In contrast to adenine, an injection of methionine alone 8 hr after the injection of ethionine did not lead to the restoration of the normal nucleolar configuration. Although nucleolar reconstruction comparable to that seen following adenine was present in a few cells (Fig. 23), the majority showed a distinctly different nucleolar appearance. The fragmentation of the nucleolus that had been observed at 8 or 12 hr after the administration of ethionine was no longer present. The margination of chromatin along the nuclear membrane and chromatin clumps in the nucleoplasm remained in many cells. There were changes suggestive of partial nucleolar reconstruction. The simplest form was a thin, rodlike structure with a diameter of approximately 0.2μ (Fig. 15). This structure usually consisted of both particulate and

fibrillar components. Frequently, two or three such structures appeared in the same nucleus. In some cells, there was further elongation of this rod-shaped structure which gave a ropelike appearance with twists or bends (Figs. 17, 18). Furthermore, in certain cells, coalescence of these structures occurred to give a more organized form (Figs. 19–21). As can be seen in Fig. 20, the thin, rope-like structure encircles several cavities which have an appearance similar to, although larger than, that of the nucleolar vacuoles of the normal nucleolus. In some other cells, the nucleoli were more compact although they exhibited an obvious, twisted, ropelike structure. In these areas the nucleolar vacuoles stand out more clearly than usual (Fig. 23).

The variability of nucleolar organization after methionine administration made it impossible to categorize its over-all structure. We have designated the nucleolar structure in general after methionine administration as a "simplified form."

Effect of Adenine 2 Hr After Its Administration, Given 8 Hr After Ethionine

When rats were given adenine alone 8 hr after the injection of ethionine and were sacrificed 2 hr later, the nucleoli appeared essentially the same as those seen 4 hr after the administration of methionine (Figs. 16, 22, 24).



FIGURES 17-21 Various simplified structures of hepatic cell nucleoli in rats given methionine 8 hr after the ethionine injection and sacrificed 4 hr later. Elongation of the rodlike structure with twisting is evident. The structure encircles irregular spaces probably corresponding to nucleolar vacuoles (*va*). *f*, fibrillar, and *p*, particulate elements. Fig. 17, $\times 18000$; Fig. 18, $\times 17500$; Fig. 19, $\times 18000$; Fig. 20, $\times 19600$; Fig. 21, $\times 24500$.

Effect of Adenine or Methionine

Administration Alone

No abnormalities of nucleolar structure were seen 4 hr after the administration of adenine or methionine.

DISCUSSION

It is evident from this study and the recent work of Miyai and Steiner (17) that the administration of ethionine to female rats induces, within a matter of hours, a series of distinct and reproducible alterations in the structure of nucleoli of hepatic

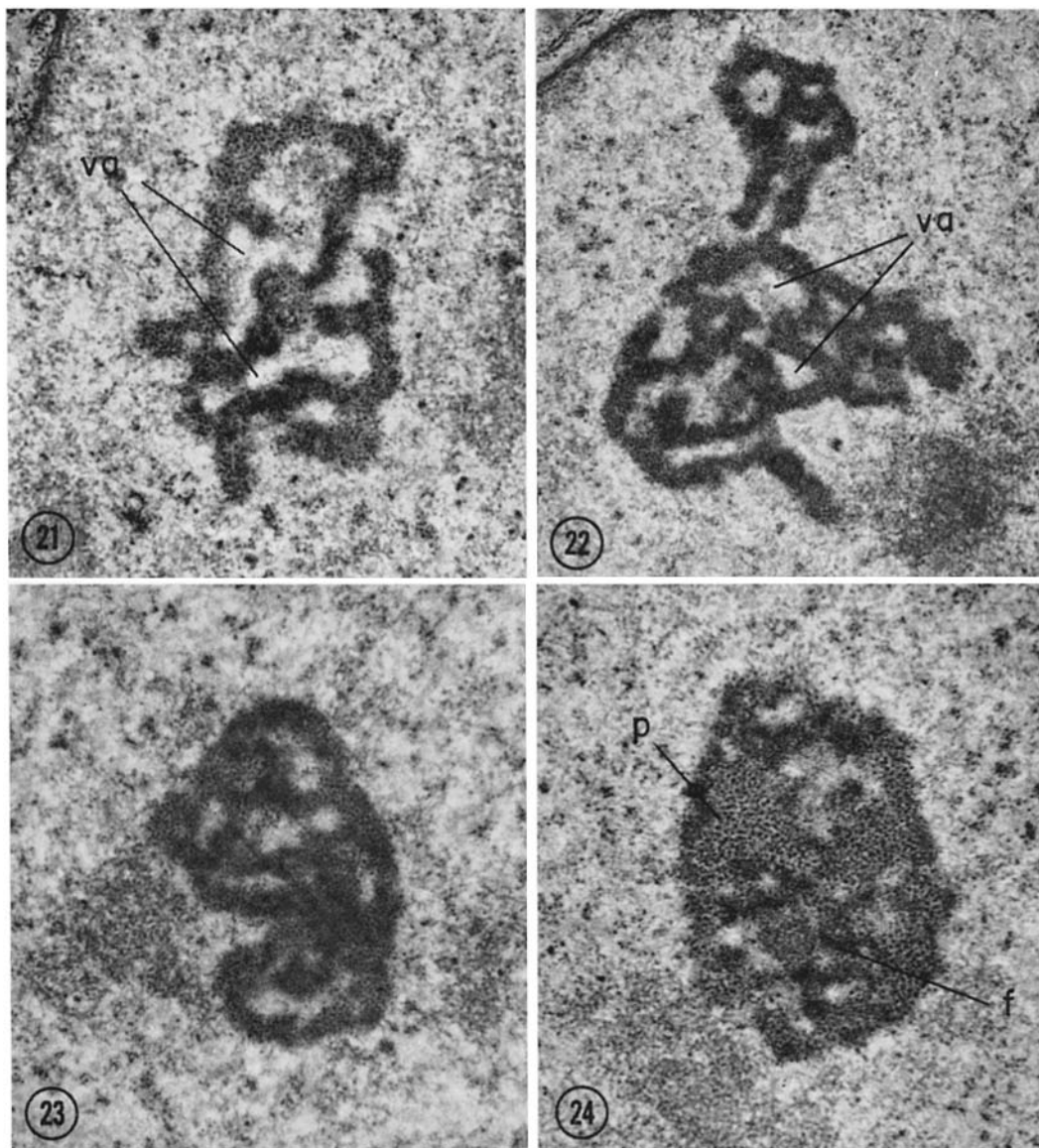


FIGURE 21 See legend under Figs. 17-21.

FIGURE 22 Hepatic cell nucleolus of rat given adenine 8 hr after the ethionine injection and sacrificed 2 hr later. Nucleolonema arranged in skeinlike pattern with a formation of nucleolar vacuoles (va). $\times 19200$.

FIGURE 23 Hepatic cell nucleolus of rat given methionine 8 hr after the ethionine injection and sacrificed 4 hr later. Nucleolonema became very compact. $\times 22800$.

FIGURE 24 Hepatic cell nucleolus of rat given adenine 8 hr after the ethionine injection and sacrificed 2 hr later. Over all appearance of nucleolus closely resembles the normal structure. Particulate (p) and fibrillar (f) components are intermingled with rather indistinct nucleolar vacuoles. $\times 25200$.

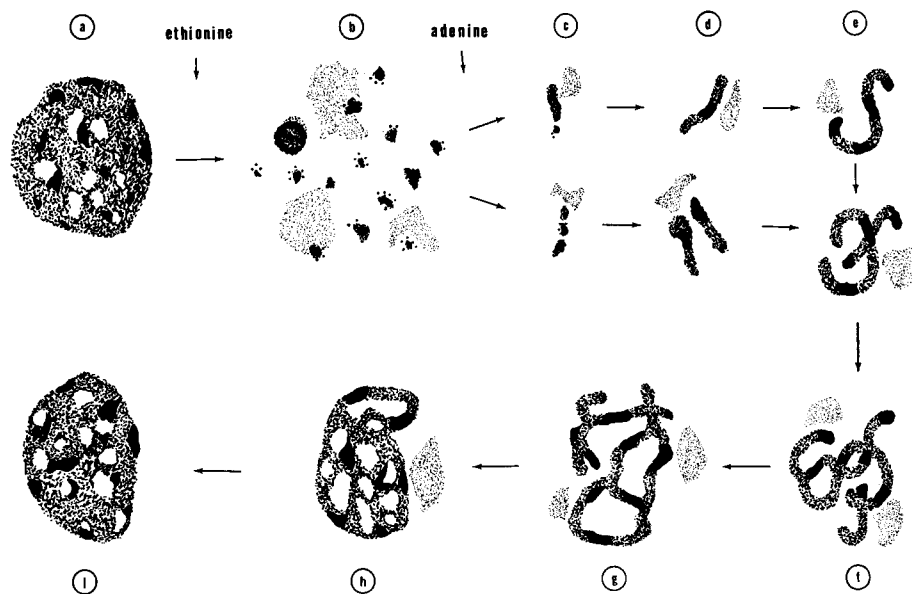


FIGURE 25 Schematic illustration of the sequential changes of the nucleolar reformation after adenine administration. *a*, normal nucleolus. *b*, disorganization and fragmentation of nucleolus after the ethionine injection. *c-h*, various structural forms encountered two hours after adenine administration. *i*, structure close to normal nucleolus encountered 4 hr after adenine administration.

parenchymal cells. These changes, which can be observed in virtually every hepatocyte and in all portions of the liver lobule, culminate in extensive fragmentation and disorganization which leave little that morphologically resembles the intact nucleolus. The development of these changes is entirely prevented by the administration of adenine or methionine even 4 hr after ethionine injection. Also, despite the extensive fragmentation of the nucleolus seen 8 hr after ethionine injection, administration of adenine at this time is followed by virtually complete restoration of normal nucleolar structure within 4 hr. Methionine administration at 8 hr is capable of substantial, but less complete, reversal of the ethionine-induced nucleolar fragmentation. The rodlike structures which we have designated the simplified form are present 2 hr after adenine injection and in many cells 4 hr after methionine administration (Figs. 15, 16). These structures consist of fibrillar and granular material and may represent a fundamental unit in the reconstitution of the nucleolonema. The fact that they are present at an early stage in adenine reversal and persist at a later time interval after methionine suggests that they represent stages in the complete restoration of normal nu-

cleolar structure, as illustrated in Fig. 25. Since the fragmentation and reformation of the nucleolus parallel fluctuations in hepatic ATP (5), it appears likely that these structural modulations are ATP dependent, as is discussed below. Whether the reconstitution of nucleolar structure is the result of reaggregation of dispersed nucleolar components or *de novo* synthesis of an entirely new organelle is not yet clear. The former proposal is intriguing in view of the stepwise fragmentation and restoration of normal structure.

The molecular basis for the nucleolar fragmentation seems to be most probably related to the induction of an ATP deficiency by ethionine. This analogue of methionine is known to have at least four major metabolic properties, all closely related to methionine (18, 19). (*a*) It can substitute for the whole methionine molecule, e.g., be incorporated into protein in place of methionine. (*b*) It can substitute for methionine in its *S* activation to form *S*-adenosylethionine. This, in turn, can participate in transalkylation reactions to form, on a selective basis, ethyl derivatives in place of methyl, e.g., formation of triethylcholine, ethylated transfer RNA, etc. (*c*) It can competitively inhibit many of the metabolic reactions of methionine and

thereby produce relative deficiencies of normal metabolites, e.g., choline, etc. (*d*) It can induce a rapid decrease in cellular ATP concentration in those cells which can form *S*-adenosylethionine (Aet) faster than they can utilize the Aet in trans-ethylation and other reactions (5). The first three mechanisms are prevented by methionine but not by adenine or other ATP precursors (18, 20). In addition mechanisms (*a*) and (*b*) are not rapidly reversed by the administration of methionine. The abnormal metabolites are destroyed relatively slowly. In contrast, the fourth mechanism is prevented by either methionine or ATP precursors and is more rapidly and efficiently reversed by the latter than the former (5). We conclude, therefore, that nucleolar alterations induced by ethionine are due to the fourth mechanism, since they are prevented by either methionine or adenine and are more readily reversed by adenine than by methionine.

The hepatic ATP deficiency induced by ethionine has many metabolic consequences as would be expected from the important role played by ATP in the control of cell economy. Among these consequences is the marked inhibition of both RNA and protein synthesis. The inhibition of protein synthesis appears to operate predominantly at the level of the cytoplasmic ribosomes (9). In view of the important roles of the nucleus and nucleolus in regulation of RNA synthesis, it is conceivable that the structural alterations seen in response to the ATP deficiency may be mainly a reflection of a disturbance in this phase of cell metabolism. The reverse hypothesis, that the structural alterations are a consequence of the ATP deficiency and that it is the nucleolar disorganization which leads to the reduction of RNA synthesis, cannot be excluded although the inhibition of RNA synthesis reaches a maximum considerably before (2–3 hr) the fully developed nucleolar changes are visible.

It is now well documented that several compounds known to combine with DNA and thereby to interfere with RNA synthesis induce characteristic alterations in the structure of the nucleolus. Actinomycin-D (21–27), aflatoxin (28, 29), mitomycin C (30), 4-nitroquinoline-*N*-oxide (31, 32), and certain other compounds (33, 34), all induce very similar nucleolar lesions consisting of a dissociation and segregation of the normal nucleolar components. It has been suggested that these effects are the consequence of the inhibition of

RNA synthesis (25, 26). Since ethionine administration induces a marked inhibition of RNA synthesis and yet does not produce precisely the same nucleolar alteration observed with these agents which combine directly with DNA, it is possible that the nucleolar dissociation and segregation they produce are not the consequence of inhibition of RNA synthesis *per se*, but rather of the molecular interactions which lead to this inhibition. Alternatively, it is possible that a similar basic structural alteration is induced by ethionine but that it is modified significantly by some other concomitant metabolic effect of ethionine such as the low ATP concentration.

Brief comment should be made on the alteration of nuclear structure other than the nucleolus. The accentuation of chromatin pattern, which appeared in the nucleoplasm of the hepatic cells treated with ethionine, resembles the appearance of nuclei of cells prepared by primary aldehyde fixation. Since we had fixed the tissue with osmium tetroxide and have not observed the changes in control animals, we consider this change to be due to the effect of ethionine. Earlier, Herman et al. (35) described margination of chromatin in the liver cells in an acute stage of ethionine intoxication. Simard (34) noted the same chromatin clumping in tissue culture cells treated with proflavin. He attributed this change to complex formation of proflavin with DNA with subsequent alteration of physicochemical properties of chromatin. In an *in vitro* study of necrosis of mouse liver, Trump et al. (36) observed the margination of chromatin around the nuclear membrane and considered the clumping of chromatin to be one of the earliest changes occurring in the cellular degenerative process. It remains unclear whether this clumping of chromatin is due to the specific effect of ethionine on the DNA portion of chromatin, or is a nonspecific cellular change altering the staining and fixation characteristics of chromatin. An increase in the number of interchromatinic granules and cluster formation becomes apparent after ethionine administration (Fig. 6). Certain other agents are reported to induce similar changes of interchromatinic granules (32, 34). Since the exact nature of these granules in their normal condition is not known, the significance of these findings remains unclear.

As judged by the developments in our knowledge of other cell organelles, it is highly probable that a reasonable hypothesis concerning the mechanism

of synthesis of ribosomal and other RNA by the nucleolus will not be possible until a clearer insight is obtained into the organizational pattern and the chemical anatomy of the various component parts of the nucleolus. On the basis of the results of the present study, it is probable that the reproducible reformation of the various components of the nucleolus induced by adenine or methionine in the ethionine-treated animal may be one model system for the analysis of some features of nucleolar structure. Hopefully, the further study of the molecular pathology of the lesions induced by ethionine and by other agents may give us new insight into one phase of the structure of the nucleus and into the response pattern of nucleolar organization to selected metabolic injury.

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REFERENCES

1. STEKOL, J. A., U. MODY, E. BEDRAK, S. KELLER, and J. PERRY. 1960. *Federation Proc.* **19**:37.
2. SHULL, K. H. 1962. *J. Biol. Chem.* **237**:PC 1734.
3. VILLA-TREVINO, S., K. H. SHULL, and E. FARBER. 1963. *J. Biol. Chem.* **238**:1757.
4. BARTELS, H., and H. J. HOHORST. 1963. *Biochim. Biophys. Acta.* **71**:214.
5. SHULL, K. H., J. MCCONOMY, M. VOGT, A. CASTILLO, and E. FARBER. 1966. *J. Biol. Chem.* **241**:5060.
6. VILLA-TREVINO, S., K. H. SHULL, and E. FARBER. 1963. *Federation Proc.* **22**:237.
7. VILLA-TREVINO, S., K. H. SHULL, and E. FARBER. 1966. *J. Biol. Chem.* **241**:4670.
8. VILLA-TREVINO, S., and E. FARBER. 1962. *Biochim. Biophys. Acta.* **61**:649.
9. BAGLIO, C. M., and E. FARBER. 1965. *J. Mol. Biol.* **12**:466.
10. VILLA-TREVINO, S., E. FARBER, T. STAEHELIN, F. O. WETTSTEIN, and H. NOLL. 1964. *J. Biol. Chem.* **239**:3826.
11. FARBER, E., K. SHULL, J. M. MCCONOMY, and A. E. CASTILLO. 1965. *Biochem. Pharmacol.* **14**:761.
12. LUFT, J. H. 1961. *J. Biophys. Biochem. Cytol.* **9**:409.
13. KARNOVSKY, M. J. 1961. *J. Biophys. Biochem. Cytol.* **11**:729.
14. MIYAI, K., and J. W. STEINER. 1965. *Exptl. Mol. Pathol.* **4**:525.
15. BRUNI, C., and K. R. PORTER. 1965. *Am. J. Pathol.* **46**:691.
16. Committee on Nucleolar Nomenclature. 1966. The nucleolus its structure and function. *Natl. Cancer Inst. Monograph* **23**. 573.
17. MIYAI, K., and J. W. STEINER. 1967. *Lab. Invest.* **16**:677.
18. FARBER, E. 1963. *Advan. Cancer Res.* **7**:383.
19. STEKOL, J. A. 1963. *Advan. Enzymol.* **25**:369.
20. GORDON, L. S., and E. FARBER. 1965. *Arch. Biochem. Biophys.* **112**:233.
21. JOURNEY, L. J., and M. N. GOLDSTEIN. 1961. *Cancer Res.* **21**:929.
22. STEVENS, B. J. 1964. *J. Ultrastruct. Res.* **11**:329.
23. JACOB, J., and J. L. SIRLIN. 1964. *J. Ultrastruct. Res.* **11**:315.
24. JÉZÉQUEL, A. M., and W. BERNHARD. 1964. *J. Microscop.* **3**:279.
25. REYNOLDS, R. C., P. O'B. MONTGOMERY, and B. HUGHES. 1964. *Cancer Res.* **24**:1269.
26. SCHOEFL, G. I. 1964. *J. Ultrastruct. Res.* **10**:224.
27. HEINE, U., A. J. LANGLOIS, and J. W. BEARD. 1966. *Cancer Res.* **26**:1847.
28. BERNHARD, W., C. FRAYSSINET, C. LAFARGE, and E. LEBRETON. 1965. *Compt. Rend.* **261**:1785.
29. SVOBODA, D., H. GRADY, and J. H. HIGGINSON. 1966. *Am. J. Pathol.* **49**:1023.
30. LAPIS, K., and W. BERNHARD. 1965. *Cancer Res.* **25**:628.
31. REYNOLDS, R. C., P. O'B. MONTGOMERY, and D. H. KARNEY. 1963. *Cancer Res.* **23**:535.
32. LAZARUS, S. S., V. G. VETHAMANY, S. H. SHAPIRO, and D. AMSTERDAM. 1966. *Cancer Res.* **26**:2229.
33. SIMARD, R., and W. BERNHARD. 1966. *Intern. J. Cancer.* **1**:463.
34. SIMARD, R. 1966. *Cancer Res.* **26**:2316.
35. HERMAN, L., L. EBER, and P. J. FITZGERALD. 1962. Electron Microscopy: Fifth International Congress on Electron Microscopy Held in Philadelphia, Pennsylvania, August 29th to September 5th, 1962. S. S. Breese, Jr., editor. Academic Press Inc., New York. **2**:VV-6.
36. TRUMP, B. F., P. J. GOLDBLATT, and R. E. STOWELL. 1965. *Lab. Invest.* **14**:1969.