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EARLY EFFECTS OF PYRROLIZIDINE ALKALOIDS ON THE FINE STRUCTURE OF RAT LIVER CELLS

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During a study of the ultrastructural effects of several hepatocarcinogens and comparison of the morphologic evolution of cell changes during tumor induction, prominent nuclear abnormalities due to pyrrolizidine alkaloids were observed in rat liver cells. The structural abnormalities resembled those due to actinomycin and other agents which suppress RNA and protein synthesis [1-9] and appeared to represent a morphologic consequence of such reduced synthesis.

With few exceptions, most previous reports dealing with the ultrastructural changes due to carcinogens and other hepatotoxins have emphasized cytoplasmic responses. While not discounting the possible relevance and importance of changes in the cytoplasm in neoplasm and other forms of cell injury, it is equally important to consider nuclear abnormalities because of the critical role of the nucleus in governing many aspects of cell metabolism. Furthermore, since normal function of the nucleolus is vital in the formation of RNA and the transmission of information from the nucleus to the cytoplasm for cytoplasmic ribosomal assembly and protein synthesis [10,11] ultrastructural alterations in the nucleolus are of considerable importance.

The pyrrolizidine alkaloids, esters of a basic alcohol containing a pyrrolizidine group, are toxic to man and animals and possess unusual properties which are useful in the study of cell biology. In experimental animals, they differ from most hepatotoxic and carcinogenic substances

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in that they are capable of causing severe, chronic and progressive liver disease after a single dose.[12–16] In addition, they cause a number of cellular changes which differ from the well-known alterations provoked by more familiar hepatotoxic agents. These include the formation of cellular aggregates on the lumen surface of central veins (veno-occlusive lesion), an atypical regenerative response manifested by formation of liver cells 3 to 4 times the normal size (megalocytosis) and the presence of prominent cytoplasmic globules in the acute stage of poisoning.[17] It has been suggested that the alkaloids act as alkylating agents[18] and possess mutagenic and carcinogenic properties which may be related to their effect on the genetic material of cells.[19] These features constitute veterinary and economic problems in several parts of the world where they cause fatal liver disease in grazing animals.[12,18] In human subjects, these agents have been reported to cause veno-occlusive disease, cirrhosis and Chiari's syndrome in those areas of the world, notably Jamaica and Africa, where they are given as a medicinal "bush tea" or in flour contaminated with seeds containing the alkaloid.[20–25] Whether the alkaloids exert their initial effect upon liver cells or upon the hepatic venous system has been a subject of controversy.

The present report demonstrates that the initial effects of pyrrolizidine alkaloids are in the nucleus of the liver cell. The changes occur within 30 minutes, recover by 72 hours and are accompanied by changes in RNA and protein content of the cells. Observations regarding the nature of the "inclusion globules," megalocytes and the veno-occlusive lesion as seen by electron microscopy are also presented.

Material and Methods

The animals used were male Sprague-Dawley rats weighing between 110 and 150 gm. One group of 22 rats was given a single intraperitoneal injection (80 mg per kg body weight) of lasiocarpine. The pure crystals were dissolved in water with several drops of 0.1N HCl added to improve solubility.[26] Two animals were sacrificed at 15 and 30 minutes, at 1, 2, 4, 6, 8, 12 and 24 hours and at 3 and 6 days. A second group of 22 rats was given a single dose of an aqueous extract of *Crotalaria fulva* (0.5 mg per gm body weight; this dosage was found to permit survival for 20 days, long enough for the development of the veno-occlusive lesion) by gastric tube and sacrificed at the same intervals. A third group of 8 animals was given a total dose of 3.2 LD_{50} of lasiocarpine in thrice-weekly injections and sacrificed at 9, 13, 16 and 20 weeks. This dosage had been found to be adequate for the production of megalocytosis in the shortest time, 9 weeks.

Ten rats given Purina® Chow diet and water *ad libitum* served as controls.

The pyrrolizidine alkaloids occur in several species of plants of the genera *Heliotropium, Crotalaria, Senecio* and several others. The genus *Senecio* alone includes approximately 1,200 species. Lasiocarpine, derived either from *Heliotropium lasiocarpium* or, along with heliotrine, from *Heliotropium europaeum* and *Crotalaria* have in common their content of pyrrolizidine alkaloids and a history of rather extensive investigation which has afforded useful information. The pyrrolizidine alkaloids are

alkamine esters which, upon hydrolysis, yield a nitrogen-containing moiety, a necine and a mono- or dicarboxylic necic acid. The combinations of necines and necic acids are numerous and many are hepatotoxic.

Microscopic Studies. For electron microscopy, samples of liver and pancreas were removed under light ether anesthesia and fixed in osmium tetroxide buffered with *s*-collidine and in phosphate-buffered glutaraldehyde. Tissues were dehydrated in a graded series of alcohols and embedded in Epon 812. Thin sections were cut with an LKB ultramicrotome using glass knives, stained with lead[27] or uranium and examined with an RCA 3 G microscope.

For orientation, adjacent semi-thin sections of Epon-embedded tissue were stained with aqueous azure A in an equal volume of 5 per cent sodium carbonate.

For light microscopy, portions of liver and pancreas were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin, the periodic acid-Schiff (PAS) technique, phosphotungstic acid hematoxylin (PTAH) and methyl green pyronin. Frozen sections of liver were stained for fat with oil red O.

Biochemical Studies. For determination of DNA, RNA and protein, 27 rats weighing between 104 and 220 gm were distributed as indicated in Table I. The experimental animals were sacrificed at 30 minutes, 15, 24, 48 and 72 hours after an intraperitoneal injection of 80 mg per kg lasiocarpine, and homogenates of the liver were prepared in a Potter-Elvehjem homogenizer. Nucleic acids were determined by a modification of the method of Schneider[28] using diphenylamine; protein was determined by the Lowry method.[29]

TABLE I

NUCLEIC ACIDS AND PROTEIN LEVELS IN RAT LIVER FOLLOWING INJECTION OF LASIOCARPINE (80 MG PER KG)

Group	Interval after injection	Number of animals	DNA mg/gm	RNA mg/mg DNA	Protein mg/mg DNA
1	Control	4	2.27 ±0.09	4.76 ±0.12	68.4 ±3.20
2	30 minutes	4	2.51 ±0.07	3.23 ±0.06	61.3 ±3.17
3	15 hours	8	2.81 ±0.13	3.03 ±0.13	54.96 ±2.48
4	24 hours	4	2.93 ±0.03	2.19 ±0.11	51.1 ±1.22
5	48 hours	3	2.52 ±0.24	3.53 ±0.45	65.0 ±5.47
6	72 hours	4	2.61 ±0.18	3.8 ±0.27	67.48 ±5.09

(mean ± standard error.)

RESULTS

The light microscopic changes in acute and intermediate stages of alkaloid poisoning consist of varying degrees of occlusion of hepatic vein branches with severe centrilobular congestion and necrosis. The lesions have been described in detail elsewhere[22] and only those directly related

to the present electron microscopic findings will be dealt with in this report. For descriptive convenience, the results are divided into nuclear and cytoplasmic alterations.

Nucleus

The Normal Nucleus. The normal ultrastructure of the mammalian nucleus and its variations during regeneration and after x-radiation have been reviewed by Davis [30]; the plasticity of this structure by light and electron microscopy [31,32] is well known. Although it has been shown that osmium fixation produces a reasonably faithful picture of the nucleus in living cells,[33] there is some inconsistency in terminology and disagreement regarding the chemical identity of certain nuclear and nucleolar constituents. In general, the nucleolus is compact and consists of an intimate combination of granules approximately 150 to 200 Å in diameter and fibrils 50 to 100 Å in diameter (Fig. 1). Both the granular and fibrillar portions are sensitive to RNAase.[5,34] Their proportion and configuration varies among normal and neoplastic cells. A third component sensitive to pepsin, similar in appearance to the fibrillar component but less dense, consists of irregular areas or cavities of amorphous material, probably protein. In addition to the chromatin, other constituents of the mammalian cell nucleus, and their chemical composition, have been described in the informative review published by Bernhard and Granboulan.[35]

After osmium fixation, chromatin is prominent adjacent to the nuclear membrane and, in normal cells, is present in small amounts, either within the nucleolus or associated with its periphery. The interchromatinic areas, formed mainly of protein, appear as irregular areas containing a fine fibrillar substance of low contrast. These areas also contain the interchromatin granules, possibly RNA, which may be in single or multiple clusters irregularly distributed amidst the chromatin. They are highly dense and measure approximately 200 to 250 Å in diameter. Perichromatin granules, 300 to 350 Å in diameter and separated from the chromatin by a clear rim or halo about 700 Å wide, are apparent only after acrolein, formalin or glutaraldehyde fixation and osmium postfixation or after phosphate-buffered osmium fixation according to Millonig's method. Their chemical identity is uncertain.

The Nucleus after Administration of Pyrrolizidine Alkaloids. Because current information regarding nuclear ultrastructure in pathologic conditions is limited, morphologic interpretation is tentative. The changes due to pyrrolizidine alkaloids primarily involved the nucleolus and the interchromatin granules. The following description, therefore, emphasizes these structures. It should be noted that the entire complex of nuclear alterations occurred throughout the hepatic lobules with no

apparent zonal predilection. The same changes occurred in Kupffer cell nuclei but not in the nuclei of vascular endothelium or ductal epithelium. The effects of *Crotalaria* extract were indistinguishable from those due to lasiocarpine and were similar in tissues fixed either in osmium or in glutaraldehyde.

The first alteration in liver cells occurred consistently within 30 minutes after the administration of either lasiocarpine or *Crotalaria* extract and consisted of a distinct separation of the fibrillar and granular components in the nucleolus (Fig. 2). By 2 hours, many nucleoli were reduced in size and consisted only of the fibrillar component, a change accompanied by reduced nuclear pyroninophilia by light microscopy. At this interval there were also prominent aggregates of granules in the nucleoplasm at some distance from the nucleolar remnants (Fig. 3). These aggregates resembled interchromatin granules in size. Their presence in liver tissue fixed with collidine-buffered osmium and the absence of a halo about them in glutaraldehyde-fixed tissue are characteristics compatible with interchromatin granules.

In many cells at 4 to 8 hours there was further separation and condensation of the granular and fibrillar components to form two adjacent zones (Fig. 4). At this time, several other morphologic variations were also present (Figs. 5 to 8).

By 24 hours the nucleoli of virtually all parenchymal and Kupffer cells were abnormal but there was marked variation in structural configuration and strict temporal reconstruction of the sequence and severity of nucleolar changes was not possible. A typical example is illustrated in Figure 9. Here the fibrillar component predominates and is associated with dense aggregates of granular material condensed at its periphery. In many nuclei the nucleolar remnants consisted of a highly dispersed granular constellation lacking specific zonal arrangement or condensed areas (Fig. 10). In other nucleoli, peripheral "satellite" granules were prominent (Fig. 11).

By 72 hours, the liver cell nuclei in rats given a single dose of either alkaloid were normal and remained so throughout the rest of the experimental period. At 9 weeks animals injected with a total dose of 3.2 LD_{50} showed megalocytosis of liver cells. In most of the enlarged cells, the nuclei were enlarged 3 to 4 times the normal diameter and, by electron microscopy, showed many complex intranuclear invaginations of cytoplasmic material (Fig. 12). Nucleoli, however, did not appear conspicuously abnormal.

Cytoplasmic Changes

Cytoplasmic Globules. In livers of animals given a single dose of lasiocarpine or *Crotalaria* extract, several round, homogeneous eosino-

philic bodies appeared in the cytoplasm at 12 hours. The bodies were PAS-positive and, not infrequently, 2 or 3 were present in a single cell (Fig. 13). Occasionally they occupied most of the cytoplasm. By electron microscopy, the bodies consisted of dense masses of cytoplasmic material limited by a single membrane (Fig. 15).

Vascular Lesions. Five days after a single oral dose of *Crotalaria* extract, large cells lined the lumen surface of central veins. Centrilobular necrosis with extravasation of red cells and partial destruction of the vein walls were also prominent at this stage (Fig. 17).

Megalocytosis. By 9 weeks liver cells in animals given 3.2 LD_{50} of lasiocarpine were markedly enlarged (Fig. 14). By electron microscopy, there was no constant or specific ultrastructural feature which distinguished these megalocytes. Most of them contained collections of numerous vesicles of smooth endoplasmic reticulum and their mitochondria were more variable in size and shape than in normal cells (Fig. 16). There was wide variation in ultrastructural appearance, even among adjacent cells and, though small cytosomes were present, other features indicative of cellular degeneration could not be discerned. The ultrastructural features of megalocytes remained essentially unchanged at 20 weeks.

The pancreas appeared normal by light and electron microscopy at all stages in all animals given either alkaloid.

Biochemical Studies

Results of biochemical studies are shown in Table I. While the average DNA values of treated animals did not differ significantly from the controls, the RNA and protein values began to fall 30 minutes after injection. Both values continued to decrease and reached their lowest value at 24 hours, when nuclear damage appeared most severe. Protein values returned to normal by 72 hours, at which time nuclear structure was also restored to normal. Similarly, RNA decreased to its lowest value at 24 hours and thereafter began to rise but did not reach the control value at 72 hours. Preliminary study of RNA and protein 8 days after a single injection of lasiocarpine indicated that recovery was sustained.

Discussion

The sequence of histologic changes in rat liver following poisoning with pyrrolizidine alkaloids has been described by several investigators but there is little agreement regarding the primary site of injury.[18,25,36,37] Because of the relatively early appearance of vascular lesions in central and sublobular veins, many workers have regarded the vessels as the

primary sites of injury.[38,39,40] In contrast, others have considered that initial toxic damage was to parenchymal cells.[41,42] It is clear from the present ultrastructural studies that the first evidence of injury was in the nucleus of parenchymal cells and that vascular changes occurred several days later. The early and consistent nuclear abnormalities throughout the lobule indicated direct interference with the metabolism of liver cells. Since the nuclear abnormalities observed in parenchymal and Kupffer cells were not observed in vascular endothelium, it is doubtful whether they were related to the veno-occlusive lesion. Similarly, the relationship of the nuclear alterations to chronic and progressive liver disease which may occur after one dose of the alkaloids is not clear at the present time.

The identical nuclear response to both lasiocarpine and *Crotalaria* extract indicated that the effects were not limited to a single alkaloid and suggests that similar alterations may be produced by other members of the pyrrolizidine group. It is apparent also that the changes were not related only to osmium fixation since they were also present in tissue fixed in glutaraldehyde.

Relationship of Pyrrolizidine effects on the Nucleus to Other Hepatotoxic Agents

The biologic effects of lasiocarpine and *Crotalaria* extract and the nuclear abnormalities bear considerable resemblance to the properties of such agents as actinomycin[5,7,8] 4-nitroquinoline N-oxide[8,43] and mitomycin (Lapis, cited by Stevens[1]) which are known to inhibit RNA synthesis. For example, lasiocarpine N-oxide[19] like actinomycin D[4,7] is capable of inhibiting hepatic regeneration. Similarly, nuclear abnormalities induced by actinomycin appear within 30 minutes[8] and recover within 2 days.[4,7] Morphologically, however, although a strong resemblance exists, the alterations induced in the fine structure of the nucleolus by pyrrolizidine alkaloids differ in some respects from those due to actinomycin. With actinomycin D, for example, the first change, uniformly reported[4-6] is loss of the nucleolonema, the fibrillar component of the nucleolus. Subsequently, the remaining nucleolar constituents sort out and form compact round aggregates with peripheral "nucleolar caps."[5,8] With alkaloid poisoning, on the other hand, the nucleolonema often persists and, though "nucleolar caps" are formed occasionally, in most cells the nucleolus is dispersed rather than compact, the pattern of distribution of various types of nuclear granules is more diverse and the nuclei have a more complex appearance. Also, unlike actinomycin,[6] the alkaloids do not affect the nuclei of pancreatic exocrine cells.

An even closer parallelism exists between pyrrolizidine alkaloids and aflatoxin, another naturally occurring, potent hepatocarcinogen derived from *Aspergillus flavus*. Aflatoxin causes hepatic lesions, including the veno-occlusive changes, remarkably similar to those induced with the alkaloids; like the latter, moreover, it causes persistent liver damage after a single dose.[44] Of further interest is the observation that aflatoxin causes changes in nuclear ultrastructure indistinguishable from those due to pyrrolizidine alkaloids and the changes first appear and recover during the same time intervals.[45] Aflatoxin, too, causes a decrease in RNA and protein content of liver cells.[46,47]

Biochemical Considerations

Recent reports indicate that the nucleus is a major site of RNA synthesis.[10,31,48,49] Nuclear RNA is synthesized in chromatin from DNA templates and transferred to the nucleolus for final assembly into ribosomes.[10,11] Nucleolar RNA, serving as a precursor of cytoplasmic RNA,[11] plays an important role in the transmission of information from the nucleus to the cytoplasm for cytoplasmic RNA synthesis. It would be expected, then, that disruption of nucleolar structure, as seen in the present studies, would result in derangement of RNA and protein synthesis. It is, of course, an oversimplification to relate the present nucleolar changes exclusively to RNA or protein synthesis. This is especially true in view of the studies of Smuckler, Iseri and Benditt[50] and Barker, Smuckler and Benditt[51] relating diminished protein synthesis to detachment of ribosomes from ergastoplasmic membranes—a finding that was inconspicuous in the early intervals of the present study. In addition, our studies dealt only with RNA and protein content of homogenates, without distinction between nuclear and cytoplasmic fractions, and involved only the total content of RNA and protein, not the rates of turnover. As a working hypothesis, however, it seems reasonable to suggest that the nucleolar abnormalities are related to diminished RNA and protein content. This proposal gains support from the close parallelism in the temporal sequence of structural alterations and the chemical abnormalities (Table I) and from the similarity of the alterations caused by pyrrolizidine alkaloids and those seen with aflatoxin and other agents known to reduce cellular RNA and protein. While it might be argued that the nuclear changes were nonspecific and represented only a stage in cell death, this seems unlikely since the abnormalities were present in virtually all cells in all zones of the lobule while subsequent necrosis occurred only in centrilobular areas.

Few reports of biochemical studies of rat liver poisoned with pyrrol-

izidine alkaloids are available.[22,52] From acute experiments Gallagher[53] suggested that there would be alterations in mitochondrial structure to account for related enzyme defects but our studies revealed no abnormality in the mitochondria in acute stages.

Relationship of the Present Studies to Previous Light Microscopic Observations on the Nucleus

Schoental and Magee[13] noted nuclear alterations which, by light microscopy, resembled mitoses or degenerative changes. It is quite probable that the abnormalities they observed corresponded to the abnormal nucleolar configurations reported here. To account for the long-term injury by the alkaloids, it has been postulated[13,19,54] that they act as mitotic poisons inducing mutations which are transmitted to succeeding generations of hepatic cells. Regarding these observations, we found no structural or chemical alterations in DNA content in acute stages, though an increase in the DNA content of megalocytes seems probable.[18,54]

Schoental and Magee[41] commented on the presence of intranuclear globules in rats poisoned with lasiocarpine. The invaginations of cytoplasm into the nucleus (Fig. 12) might well appear as eosinophilic globules by light microscopy. The presence of invaginations, clefts and lipid in nuclei cannot be related directly to the acute nuclear changes since they occurred several weeks after the acute changes had returned to normal.

Intracytoplasmic Globules. Several workers have discussed the nature of the intracytoplasmic globules which occur early in alkaloid poisoning. Christie[17] found that the droplets contained RNA and suggested that they represented "a degenerative product of the cytoplasm." On the other hand, Bull and co-workers[26,55] demonstrated that they were pyroninophilic and PAS- and PTAH-positive and suggested that they were pathognomonic for pyrrolizidine poisoning. By electron microscopy, the bodies consisted of compact aggregates of degenerated cytoplasmic organelles limited by a single membrane and were consistent with the process termed focal cytoplasmic necrosis or degradation[56] resulting in the formation of cytolysomes. This process has been reported in several forms of injury in a wide variety of cell types and represents a nonspecific response to injury. The main point related to alkaloid poisoning is that prominent focal cytoplasmic necrosis and, indeed, all cytoplasmic alterations, appeared several hours after the nucleolar changes.

The Vascular Lesion. Although total occlusion of central veins was not observed, prominent cellular aggregates were present on the lumen

surface of these vessels. By electron microscopy, the venous lesion did not appear to represent recanalization of a thrombus [25] since thrombotic material was never observed. The cells, instead, resembled macrophages and swollen endothelium.

Megalocytosis. Megalocytosis was another prominent feature in pyrrolizidine alkaloid poisoning. While not specific for this form of injury, the degree and uniformity of cell enlargement as well as the histochemical properties of the large cells [57] distinguished them from other forms of enlargement of hepatic cells. Their lack of ultrastructural resemblance to liver cells of animals treated with several carcinogens [58] coupled with the scarcity of mitoses, especially abnormal ones, suggested that they did not represent a pre-neoplastic condition. The abundance of comparatively normal cytoplasmic organelles and the absence of degenerative changes resembled, instead, an exaggerated regenerative response to the necrosis which occurred earlier.

Conclusions

An electron microscopic study of the early changes in rat liver following the administration of lasiocarpine or *Crotalaria* extract revealed prominent nuclear abnormalities occurring within 30 minutes after administration of the alkaloid and a return to normal by 72 hours. The nuclear changes were accompanied by a decrease in hepatic RNA and protein content and resembled the abnormalities in nuclear structure caused by other agents which suppress RNA and protein synthesis.

Nuclear alterations were followed by focal cytoplasmic necrosis and aggregation of cells resembling macrophages on the lumen surface of central veins. No single, specific ultrastructural feature distinguished the enlarged liver cells (megalocytes) which occurred 9 weeks after a total dose of 3.2 LD_{50} of lasiocarpine. The fine structural appearance of megalocytes resembled an abnormal regenerative response.

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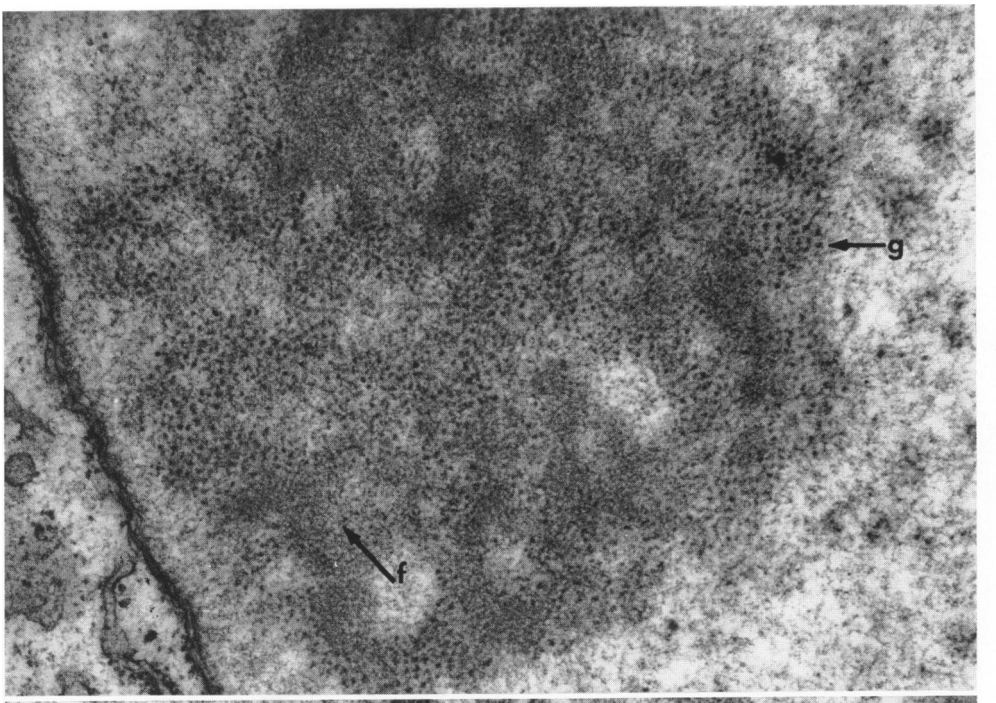
[*Illustrations follow*]

LEGENDS FOR FIGURES

All electron micrographs are from sections of rat liver stained with lead.

FIG. 1. The nucleolus of a normal liver cell. The fibrillar (f) and granular (g) components are intimately mixed in approximately equal proportions to form a rather compact, roughly circular structure. × 21,600.

FIG. 2. The nucleolus of a liver cell 30 minutes after the intraperitoneal injection of 80 mg per kg lasiocarpine. There is early separation of the fibrillar (f) and granular (g) components. The nucleolus is no longer compact and circular. × 21,600.



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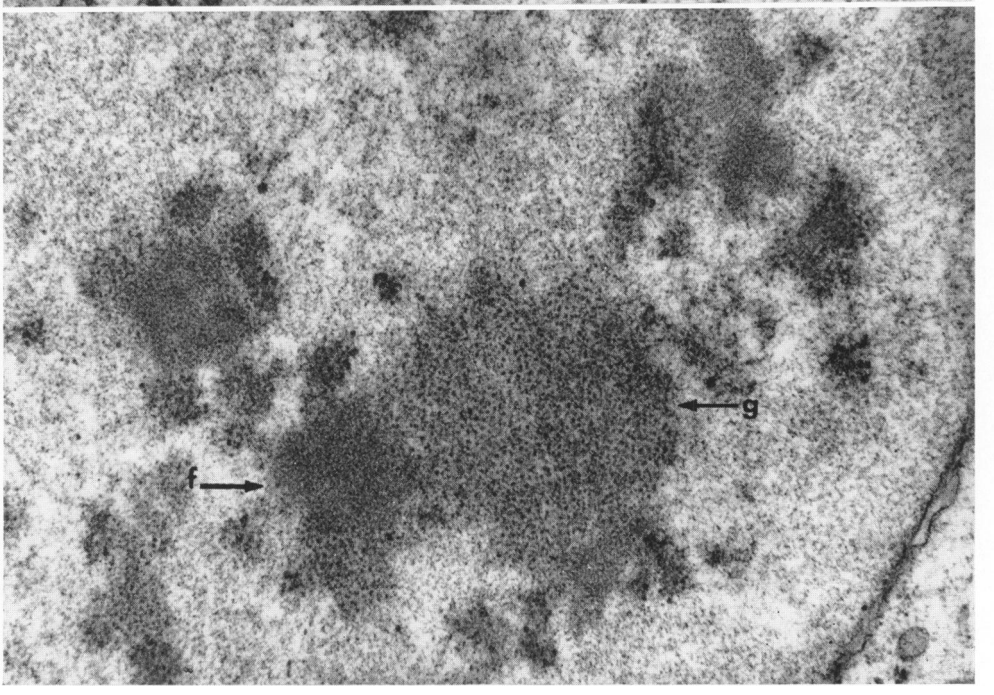

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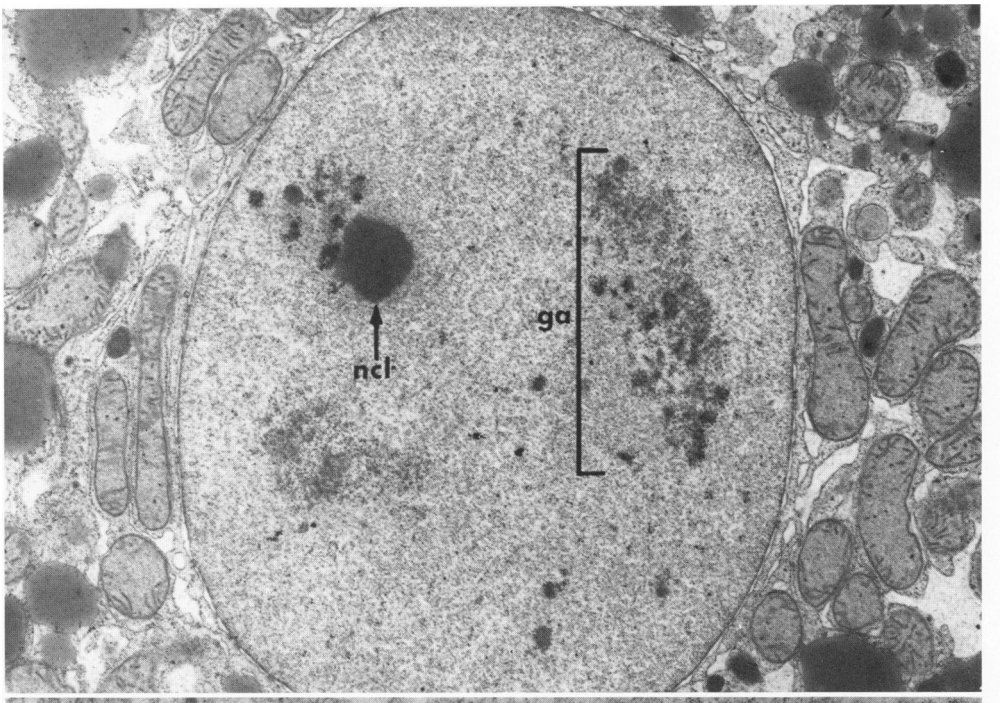
FIG. 3. Two hours after the injection of lasiocarpine. The nucleolus (ncl) is markedly reduced in size and consists predominantly of its fibrillar component. Elsewhere in the nucleoplasm are aggregates (ga) resembling interchromatin granules. × 7,000.

FIG. 4. Eight hours after the injection of lasiocarpine. There is further separation and condensation of the fibrillar (f) and granular (g) components to form two distinct zones. × 49,500.



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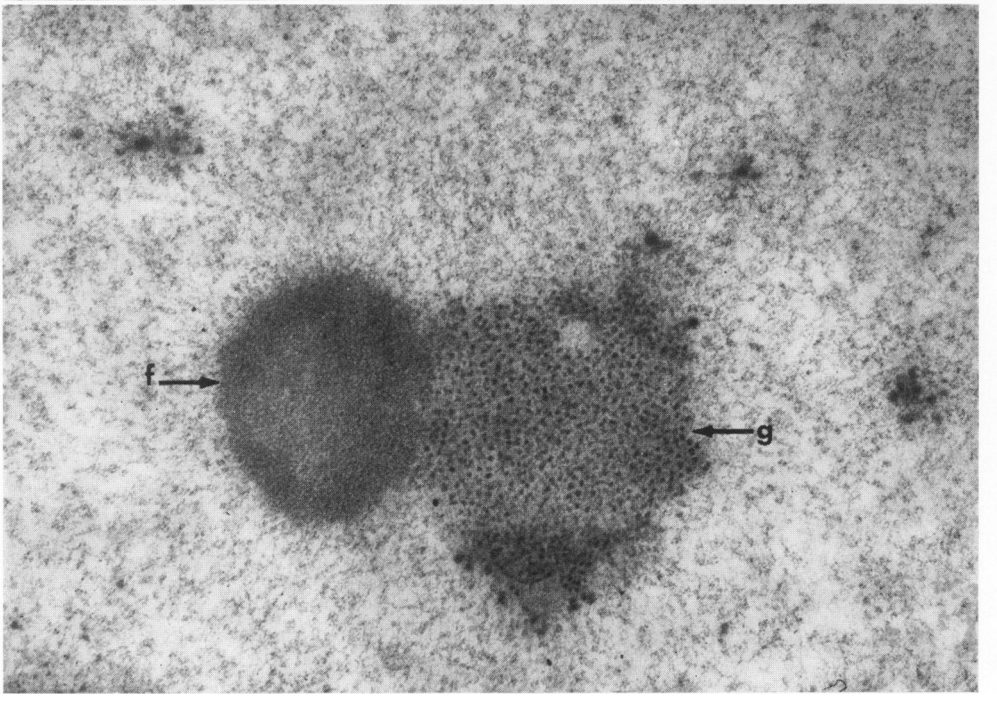

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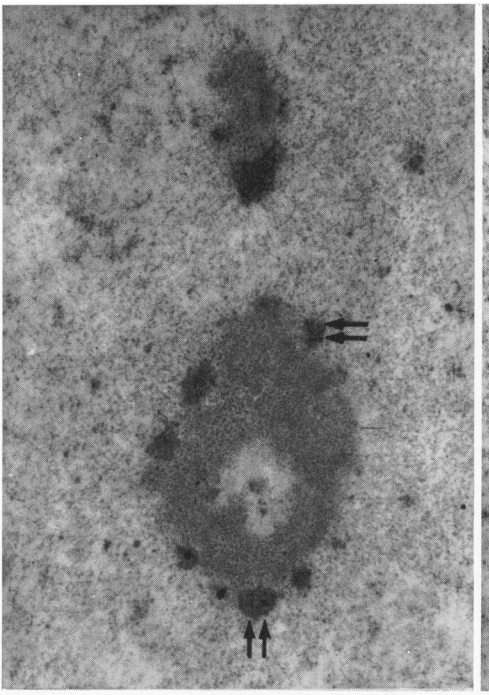
FIGS. 5 to 8. Eight hours after the injection of lasiocarpine. Several morphologic variations in nucleolar structure are manifest. Figure 5: A torus-shaped nucleolar remnant is composed of both components and exhibits an electron-lucent center. At the periphery, there are several aggregates of a third type of granule (double arrows) which are denser than the normal nucleolar constituents. Figure 6: Shown are the fibrillar and granular components from two distinct zones with the denser granules (double arrows) forming a rim at the periphery of the granular zone. Figure 7: A nucleolus exhibits three zones with the granular area sandwiched between two fibrillar areas. Figure 8: A torus-shaped remnant consists of the fibrillar component and is adjacent to a granular projection. × 21,600.



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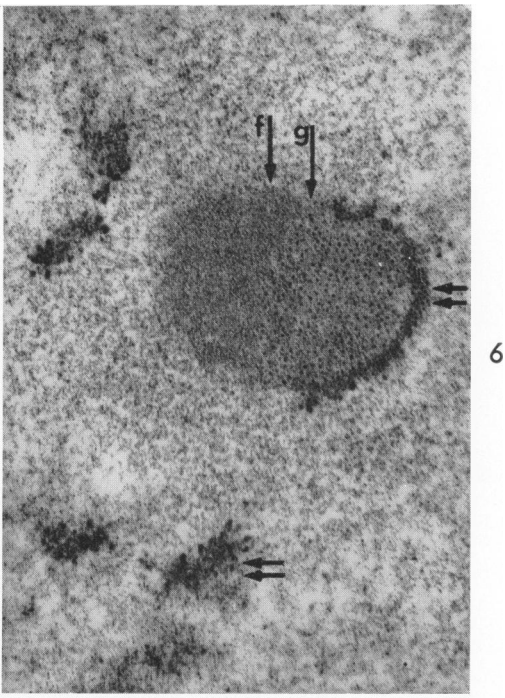

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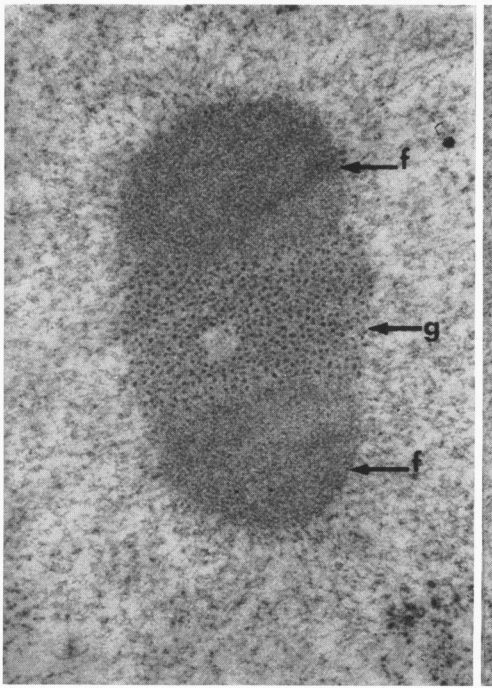

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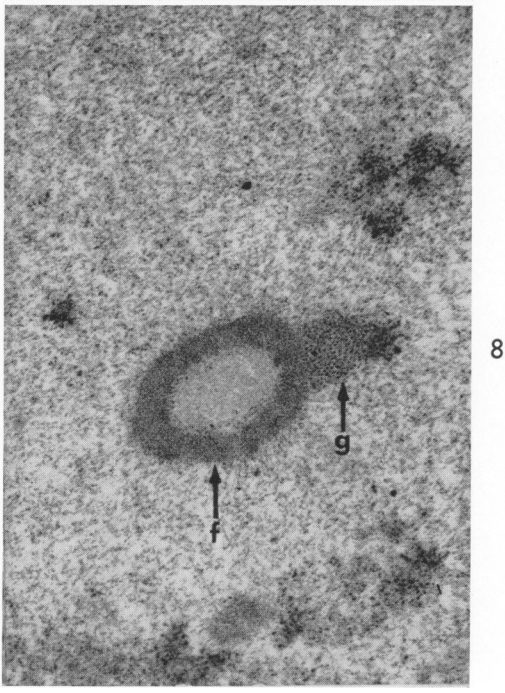

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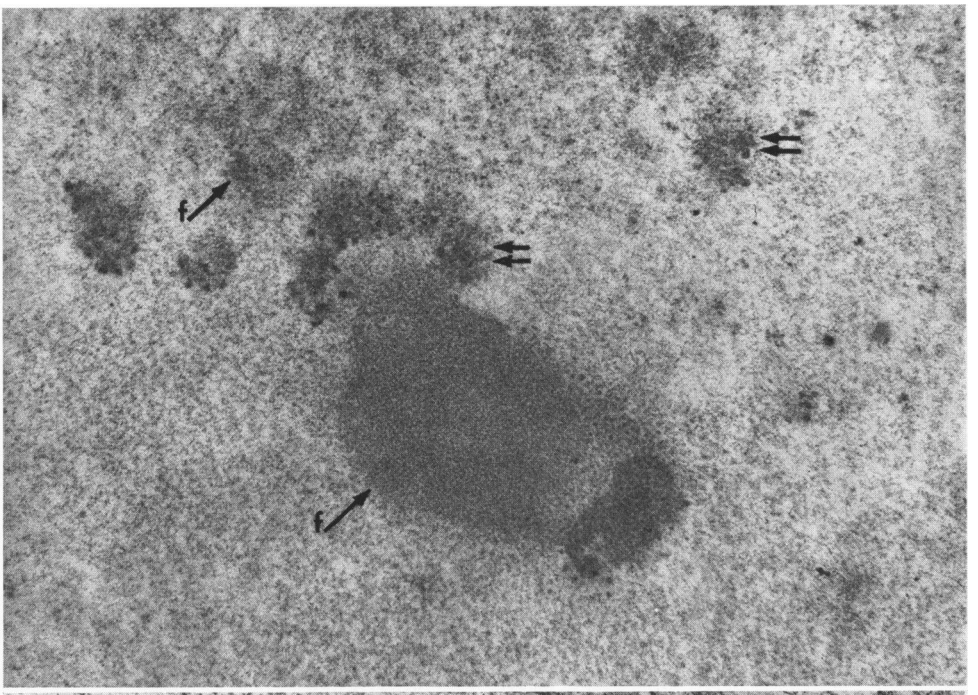

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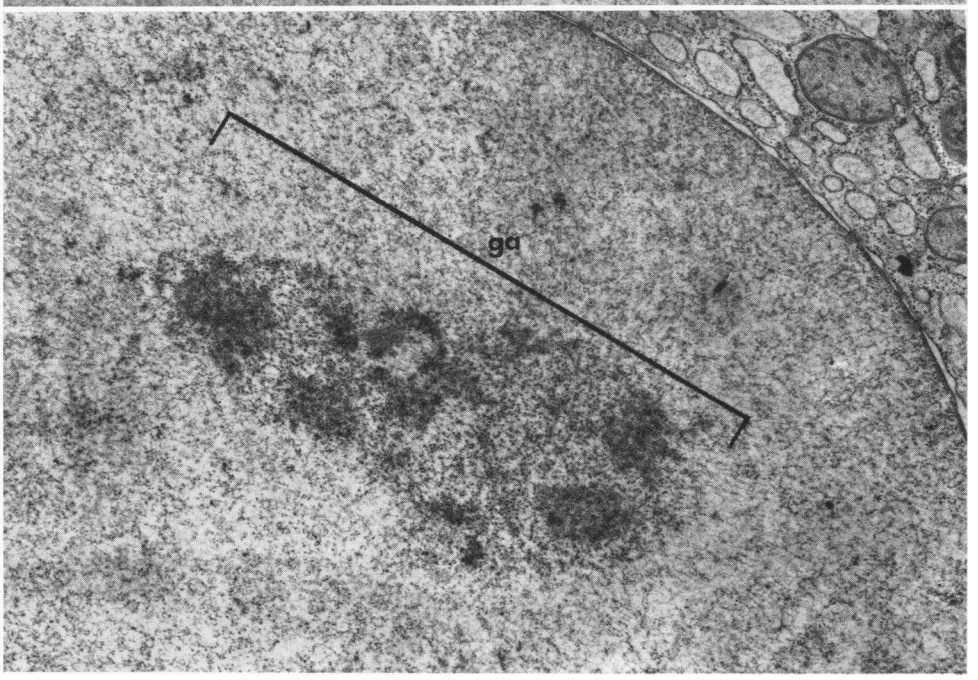

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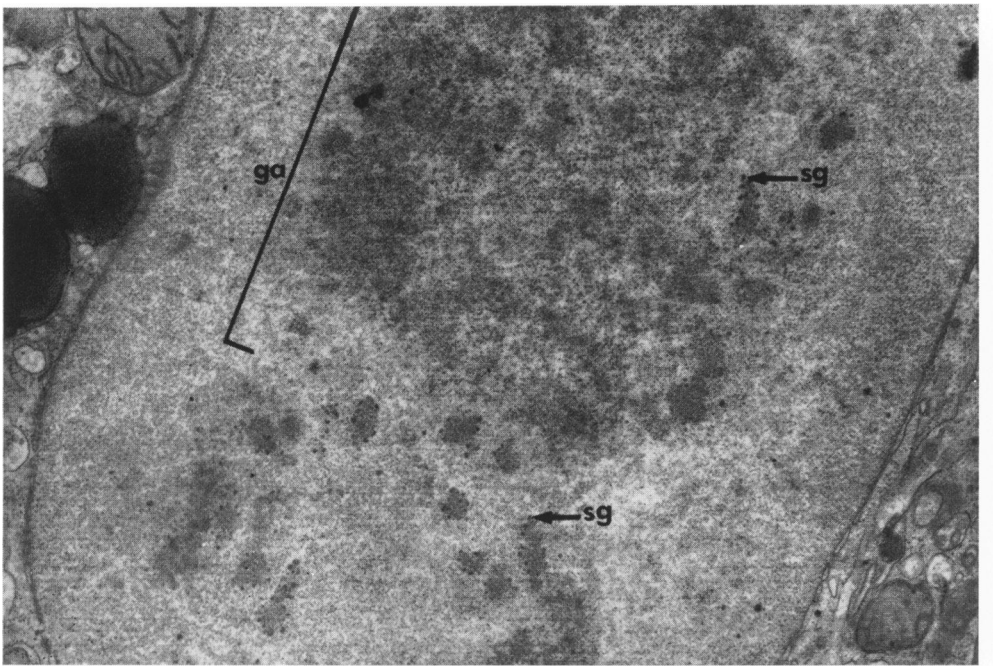

FIG. 9. Twenty-four hours after the injection of lasiocarpine. The fibrillar component predominates and is associated with aggregates of dense material at its periphery (double arrows) and at some distance away in the nucleoplasm. × 21,600.

FIG. 10. Twenty-four hours after the injection of lasiocarpine. The nucleolar remnant consists of a dispersed granular constellation lacking zonal arrangement. × 21,600.

FIG. 11. Twenty-four hours after the injection of lasiocarpine. A large granular aggregate (ga) is present. Several portions are surrounded by distinct satellite granules (sg). × 21,600.

FIG. 12. Nine weeks after the administration of 3.2 LD_{50} of lasiocarpine. The nucleus is markedly enlarged and contains several cytoplasmic invaginations (i). l = lipid; ncl = nucleolus. × 7,200.

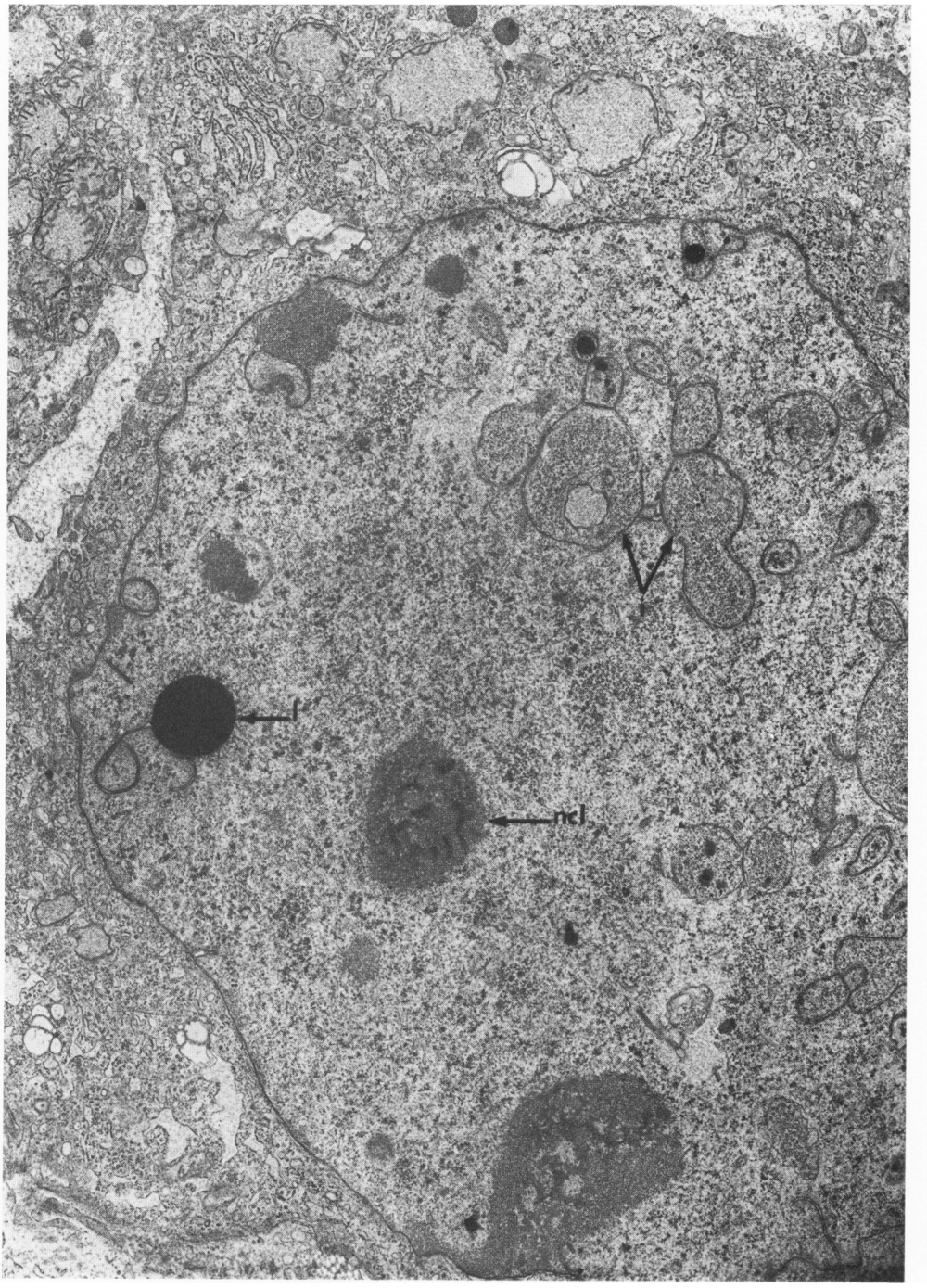

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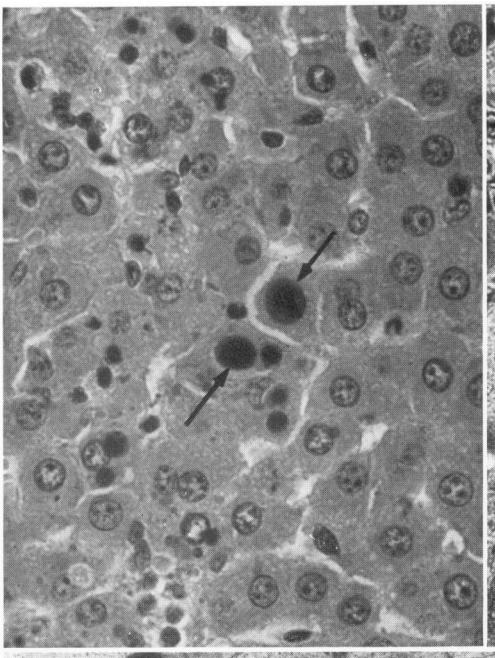
FIG. 13. Twelve hours after the injection of 80 mg per kg lasiocarpine. Round homogeneous bodies are prominent in the cytoplasm of several cells (arrows). PAS stain. × 560.

FIG. 14. Nine weeks after the administration of 3.2 LD_{50} of lasiocarpine. Megalocytosis and oval cell proliferation are apparent. The enlargement of liver cells is approximately three times greater than normal. Phosphotungstic and hematoxylin stain. × 360.

FIG. 15. Twelve hours after the administration of 80 mg per kg lasiocarpine. A cytoplasmic body illustrated in Figure 13 consists primarily of altered mitochondria (m) and lipid (l). The body is surrounded at the left by relatively uninvolved cytoplasm (cyt). × 5,700.



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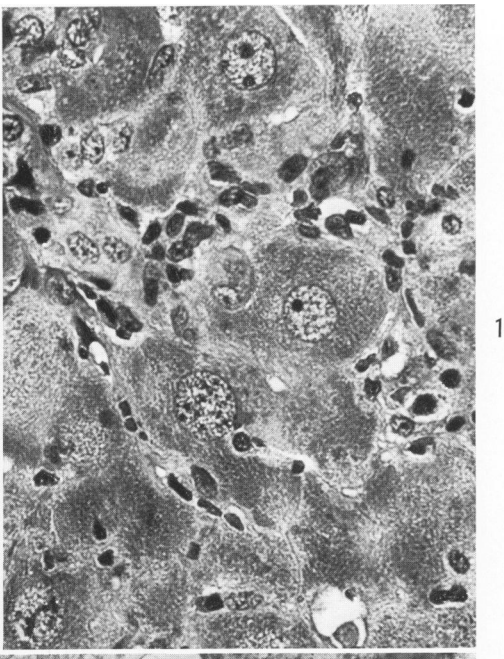

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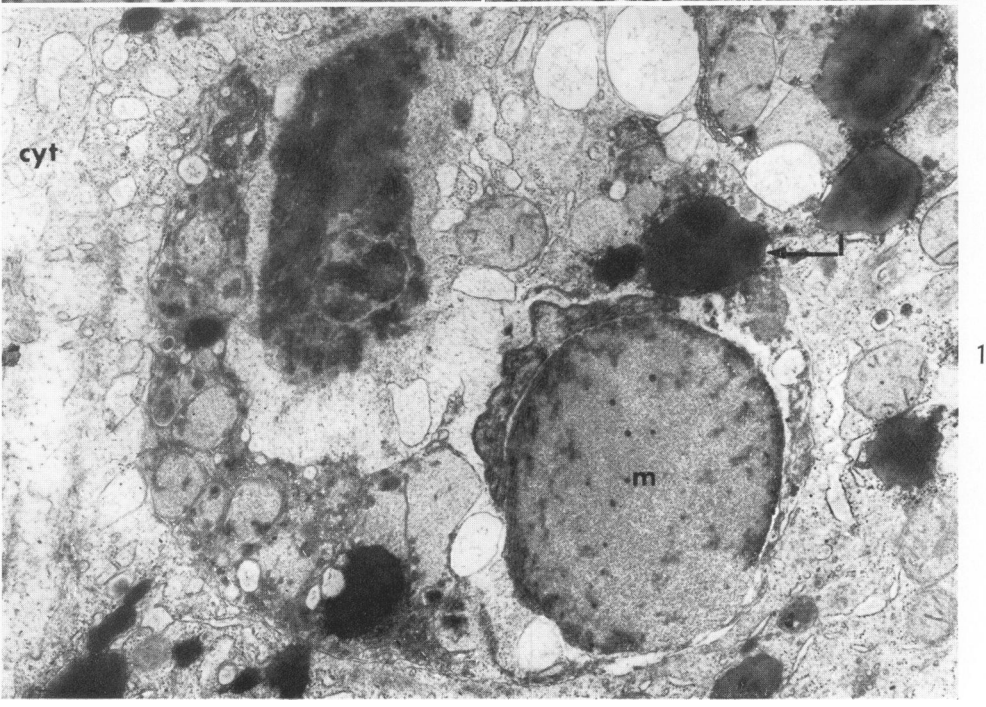

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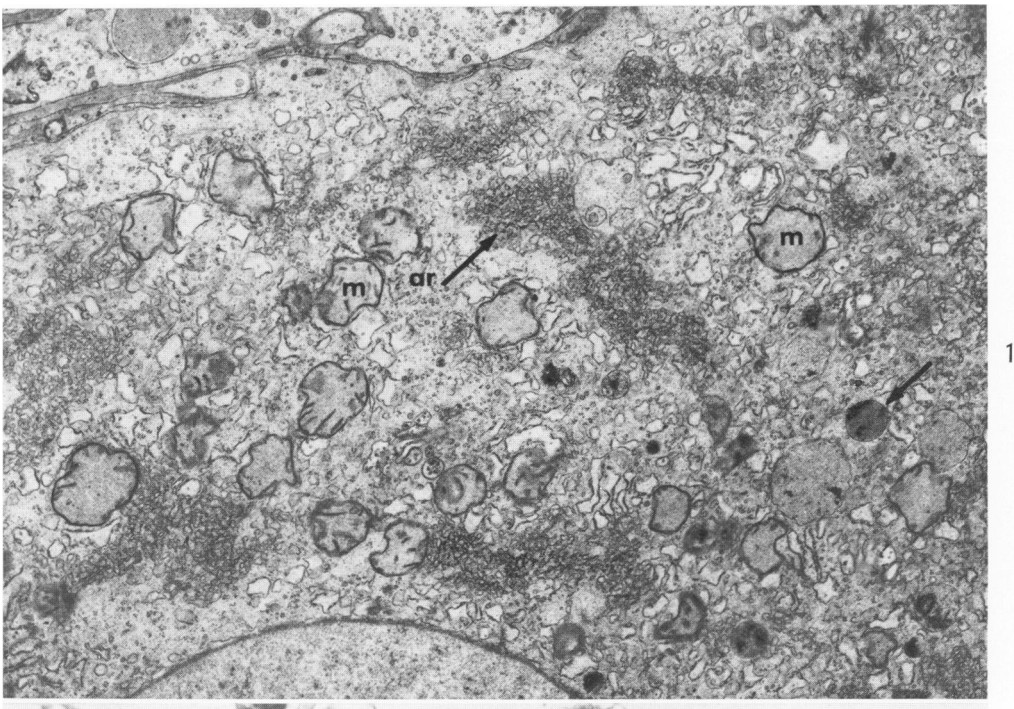
FIG. 16. Shown here are the megalocytes illustrated in Figure 14. The large cells contain numerous collections of agranular reticulum (ar). The mitochondria (m) are irregular in shape and few small cytosomes (arrow) are present. × 5,700.

FIG. 17. Five days after the oral administration of 0.5 mg per gm *Crotalaria* extract. The lumen surface of the central vein contains a collection of large cells partially occluding the lumen. Extravasated red cells amidst fragmented reticulum are apparent in the centrilobular area. The vein well is disrupted at x. Azure-sodium carbonate stain. × 530.



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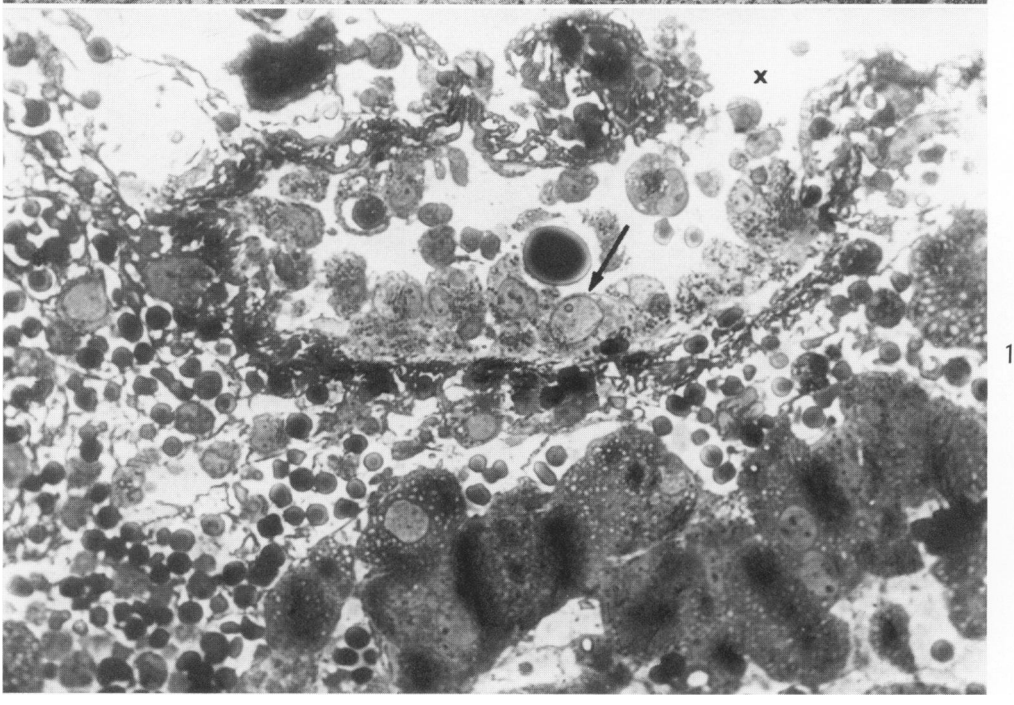

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