CELLULAR AND SUBCELLULAR EFFECTS OF IONIZING RADIATIONS

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The discovery of x-rays in 1895 by Röntgen and of radium in 1898 by the Curies was followed very shortly by an awareness of the adverse effects which these rays have on living cells. In 1902 Frieben[1] reported the first case of human cancer induced by x-rays and in 1910 Clunet[2] used x-rays to produce malignant tumors in experimental animals. The fact that the cell nucleus appeared to be the portion of the cell which was most affected by x-rays was reported as early as 1911 by Hertwig.[3] In 1923 Strangeways and Oakley[4] reported that x-rays produce a temporary arrest of mitosis followed by the production of apparently normal daughter cells. It remained for Muller[5] to understand that these nuclear effects were in part genetic in nature and to demonstrate that they involved hereditary changes in the fruit fly. The effects of x-radiation on the cytoplasm have received lesser attention than the nuclear effects. In the main, this is because these effects are more subtle and because they are considered to be nongenetic in nature. Observations on the effects of x-radiation on the nucleus as contrasted with its effects on the cytoplasm have generated a controversy over the relative importance of the responses of these two areas of the cell. This controversy has not been entirely resolved.

Few data are available in the literature which relate irradiation-induced alterations in cellular fine structure with irradiation-induced alterations in cell behavior. It is the purpose of this communication to record these two types of data on cellular and subcellular responses to irradiation and to indicate some ways in which specific observations in each area may be correlated.

MATERIAL AND METHODS

Chang liver cells grown in serial culture were transferred to cover slips or to perfusion chambers and maintained in Eagle's tissue culture medium with 10 per cent horse serum. For the x-radiation studies the cells were irradiated on the first day after subculture through the 0.18 mm. glass cover slips on which they were prepared. The

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total radiation dose of 600 r was delivered at the rate of 100 r per minute, from a 250 kv. Westinghouse x-ray machine, operating at 11 ma., at a distance of 50 cm., with filters of 1 mm. of aluminum and ½ mm. of copper. Similar cultures were irradiated with electrons by incorporating into the media sufficient P^{32}-labeled sodium phosphate to produce radiation levels of 18, 12, 5 and 0.6 mc. per cc. respectively. The isotope was obtained from the Oak Ridge National Laboratory and incorporated into an isotonic saline solution. This was combined with Eagle's medium to obtain the desired activity.

Following irradiation with 600 r and during electron irradiation, the cultures were photographed by phase contrast time-lapse cinematography. Over 1,500 hours of observations were made in experiments ranging from 6 hours to 240 hours post irradiation. Approximately 400 hours of observations were made on electron-irradiated cultures during the period of the irradiation. These films were edited, reviewed and compared with a similar number of films of unirradiated cells. After 600 r of x-radiation, additional cultures were returned to the incubator at 37.5° C. for later fixation and study. Cells from these cultures were prepared for study by electron microscopy by fixation in osmic acid buffered to pH 7.4 and embedding in methacrylate. The cells for these studies were fixed at 25 hours, 40 hours, 50 hours, 60 hours, 70 hours and 168 hours after x-radiation. A similar number of control cultures were treated in the same manner with the exception that they were not subjected to irradiation.

Results

One of the most dramatic biologic effects of moderate nonlethal doses of ionizing radiations was the dissociation of cell division from cell growth. The dissociation resulted in the production of very large mononucleated cells following exposure to electron radiation at the 0.6 mc. per cc. level of P^{32} and to 600 r of ionizing radiations (Fig. 1 to 3). The enlargement was effected by an enlargement in the size of the various cellular and subcellular structures and was accomplished while the cell maintained the normal ratio of cell size to cell organelle size. In the enlargement of the entire cell, the cytoplasmic mass and the nuclear mass enlarged. Tables I and II compare the nuclear and cytoplasmic diameters of these large mononucleated cells with the same diameters in unirradiated control cells. In the enlarged cytoplasmic mass the endoplasmic reticulum increased in amount, the mitochondria enlarged and changed shape and the Golgi zone hypertrophied. In the nucleus the nucleoli were increased in size and number. Except for their inability to divide, the giant mononucleated cells displayed normal cellular activities. The cytoplasmic membrane was widely extended and actively motile. Pinocytosis was unimpaired and the movements of the small cytoplasmic lipoprotein droplets were normal in nature, thus indicating that the consistency of the cytoplasm had not been altered by the effects of the ionizing radiations. In all cultures the cells were frequently observed to contain cytoplasmic inclusion bodies. With the phase microscope the bodies appeared irregular in size and shape and had areas of varying opti-

cal densities. Studies of the fine structure of the inclusions indicated that they had a membranous nature. In some, the bulk of the inclusion appeared to be made up of fine vesicles which resembled condensations of endoplasmic reticulum. The vesicles were intimately associated with small electron-dense particles presumed to represent agglutinated or

TABLE I

SIZE CHANGES IN CHANG LIVER CELLS FOLLOWING 600 r X-IRRADIATION (100 r PER MINUTE FOR 6 MINUTES). NUCLEAR DIAMETER OF THE UNIRRADIATED CELL EQUALS 1 UNIT

Days following 600 r x-irradiation	Nuclear diameter	Cell diameter
Pre-irradiation	1	3
1	1.5	3
2	2	5
4	3	7

TABLE II

SIZE CHANGES IN CHANG LIVER CELLS GROWING IN MEDIUM CONTAINING 0.6 mc. per cc. BETA IRRADIATION. NUCLEAR DIAMETER OF THE UNIRRADIATED CELL EQUALS 1 UNIT

Weeks in medium with 0.6 mc. per cc. beta irradiation	Nuclear diameter	Cell diameter
Pre-irradiation	1	3
1	1.3	3.7
2	1.8	5
3	2.3	6.7

altered ribosomes. In others, the membranes were larger, and the occasional appearance within them of small double membranes indicated the possibility that this type of inclusion might contain fragments of degenerating mitochondria. In still other inclusions one could trace the accumulation of lipid material from small droplets to rather large masses of osmiophilic material (Figs. 4 to 6).

With phase contrast time-lapse motion pictures, two main types of division anomalies were observed in cells receiving both types of irradiation. The frequency and nature of these anomalies were the same in the cultures which received 600 r of x-radiation and in the two lower dose levels of electron radiation, namely, 5 and 0.6 mc. per cc. respectively. The most common was the failure of the cell to complete a mitosis once it had begun and then to persist as a rounded cellular mass which eventually disintegrated. If the cell was sufficiently vigorous to complete mitosis, one abnormal end result was the production of a binucleated cell. The production of such cells was observed to involve more than one

mechanism. In one instance 24 hours after 600 r of x-radiation, a cell completed an apparently normal division up to the point of complete telophase separation. At this time a cytoplasmic bridge between the two daughter cells remained, and after a short period of time this widened. The two daughter cells then recombined across the bridge to form a binucleated cell (Figs. 7 to 10). In another instance 50 hours after 600 r, one cell divided and gave rise to a binucleated cell without any attempt at telophase separation (Figs. 11 and 12). On some occasions a binucleated cell had nuclei of unequal size. The smaller of these, the micronucleus, always contained at least one small nucleolar fragment (Figs. 13 and 14). Three days after 600 r many of the cells in the culture had become binucleated. At this time a number were observed to undergo division. Division of a cell such as this might result in the formation of two binucleated daughter cells in which each new nucleus was of equal size (Figs. 15 and 16).

In addition to the mononucleated and binucleated giant cells, multinucleated giant cells occurred following each type of irradiation. One mechanism in the formation of these giant cells was cytoplasmic fusion which took place at a point of contact between the cytoplasmic membranes of two adjacent cells. At the point of contact the two membranes appeared to merge into one another, and the smaller of the two cells was gradually incorporated into the cytoplasmic mass of the larger, thereby creating one multinucleated giant cell. This process was observed to occur most frequently between a multinucleated cell and a smaller binucleated cell. After a period of many hours the nuclei of the two original cells could not be separated or identified in the rather conglomerate pile of nuclei in the new giant cell (Figs. 17 to 19).

The effects of ionizing radiations on the subcellular organelles were important features of the radiation effects on cells. These effects could be observed by phase contrast time-lapse motion pictures and by electron microscopy. Alterations in the size and shape of mitochondria in living cells were observed by phase contrast after 600 r of x-radiation. These consisted of longitudinal splitting, the development of irregular and bulbous swellings of a localized or generalized nature, the formation of mitochondrial loops and of partial splitting to form Y-shaped mitochondria. Studies of their fine structure indicated that many of the mitochondria were branched, while many others contained localized swelling usually located at one end. In the swollen areas the cristae mitochondrialis were frequently ruptured and diminished in number (Figs. 20 to 25).

In the unirradiated cell, phase microscopy was not capable of sharply delineating the Golgi apparatus as a distinct membranous structure. It appeared as a perinuclear zone of different optical density than the surrounding cytoplasm or the adjacent nucleoplasm. It was vaguely de-

marcated from the cytoplasm but clearly separated from the nucleus by the nuclear membrane. In the irradiated cell which had undergone generalized enlargement the Golgi zone was proportionately enlarged. By electron microscopy the membranous character of the Golgi apparatus was clearly discernible. The membranes were enlarged and more numerous than normal and joined together by fine filamentous threads (Figs. 26 and 27).

In phase contrast the nucleoli of the majority of unirradiated Chang liver cells appeared as optically dense, homogeneous, smooth round masses. In a few instances unirradiated nucleoli had a slightly vacuolated appearance. By electron microscopy the common type of nucleolar fine structure was a round cluster of small particles having a moderate electron density. Scattered amongst these were slightly larger particles with somewhat greater electron density. In a small number of unirradiated cells the fine structure of the nucleolus varied from this pattern. The small nucleolar granules were arranged in a skein-like or meshwork pattern. The slightly larger, darker particles were randomly distributed within the meshwork, and occasionally formed small clusters. The nucleoli corresponded to the vacuolated nucleoli occasionally observed with the phase microscope (Figs. 28 and 29). After x-radiation the appearance of the nucleoli changed markedly. By phase microscopy they could be seen to enlarge, to fragment and to change into irregular shapes. In many instances they appeared vacuolated and several days after radiation they could be seen to coalesce to form distinct holes with sharply defined borders. By electron microscopy the alterations were observed in detail. In the first 60 hours after 600 r of x-radiation the nucleoli, in increasing numbers, assumed a mosaic pattern until 75 per cent of the cells contained nucleoli of this type. The mosaic represented an accentuation of the skein-like pattern occasionally seen in unirradiated nucleoli. In these instances the larger denser granules tended to cluster together at the edges of the masses of the smaller granules. At 60 hours this type of nucleolus tended to diminish in number and to be replaced by a nucleolus with one or two large central holes. As the nucleolar mosaic pattern disappeared, the latter "doughnut" type of nucleolus was increased in frequency until at 240 hours 60 per cent of the nucleoli exhibited such holes. Projecting from the inner and outer margins of the holes were occasional clusters of larger, more electron-dense nucleolar granules. Thus one of the features of the response of the nucleolus to sublethal radiation was an increase in vacuolation and a redistribution of electron-dense nucleolar particles. The increase in vacuolation began with the formation of small vacuoles which later coalesced to form one or several large holes (Figs. 30 to 33).

The nuclear membrane exhibited two responses to sublethal irradi-

ation. One of these consisted of a generalized enlargement accompanying the enlargement of the nucleus in the formation of the mononucleated giant cell. In addition, the nuclear membrane frequently folded upon itself so as to produce deep clefts. The folds and clefts appeared in phase microscopy as either indentations of the periphery of the membrane, or as fine linear stripes crossing the surface of the nucleus. By electron microscopy the deep folds could be clearly visualized. In the depths of the nuclear membrane folds there were nucleolar synechiae between the nucleolus and the inner lining of the membrane. The fine structure of the nuclear membrane itself did not appear to be altered (Figs. 2, 30 and 34).

In order to demonstrate some of the irradiation-induced functional and structural alterations in the nuclear and cytoplasmic membranes, extremely high levels of irradiation were required. An instantly lethal effect of electron irradiation on these cells could be achieved by adding 18 mc. per cc. of P^{32} to the culture media. By phase microscopy cells subjected to this amount of irradiation had the appearance of "fixed" cells. The nuclear membrane wrinkled; the nucleus was reduced in size, increased in optical density and, in most cases, was surrounded by a chromophobic corona. These findings indicated that the nucleus had retracted slightly from the surrounding cytoplasm. The cytoplasm of the "fixed" cells was also increased in optical density and was somewhat contracted. Nucleoli were apparently not altered. In such dead cells the peripheral membrane, the cytoplasmic particulate matter and the nucleus remained motionless. Dead cells of this type remained in the culture without lysis for many hours (Fig. 35). Following the addition of 12 mc. per cc. of P^{32} to a cell culture, a different pattern of cell death was observed. For several hours the cells functioned and appeared essentially as normal cells, following which they began to die. Death of all the cells in such a culture was then complete less than one hour after the first cell died. In this type of cell death the cytoplasm collapsed about the nucleus and in doing so, left behind numerous membranous streamers attached to the cover glass. In these spider web attachments one frequently found small trapped lipoprotein droplets. After this type of cell death, the entire cell, rather than just the nucleus, was surrounded by a chromophobic corona (Fig. 36). Still another pattern could be observed after the addition of 5 mc. per cc. of P^{32}. After several hours the cytoplasmic membranes of some, but not all, of the cells began an intense micro-bubbling phenomenon. One of the consequences of bubbling was the budding off of circular fragments of the peripheral cytoplasmic membrane. The circular fragments separated from the cell and floated free in the culture media. In some instances so many of these cytoplasmic fragments were detached

that the cell later collapsed and died. Electron micrographs of the cytoplasmic membrane in such cells showed that the cytoplasmic bubbles contained portions of endoplasmic reticulum and occasional membranous fragments suggestive of portions of mitochondria. Intact mitochondria were not observed in the micro bubbles (Figs. 37 to 39).

Discussion

Immediately lethal effects of ionizing radiations have been reported by Spear,[6] Halberstaedter and Doljanski[7] following 100,000 to 200,000 r x-radiation. These levels of radiation cannot be rapidly achieved with conventional x-ray equipment, and hence it is somewhat difficult to compare these effects with the effects of the 18 mc. per cc. of P^{32} which we observed to be virtually instantly lethal. This level of electron irradiation has the effect of fixing the cells in much the same fashion as formalin or heat. In these cells the membranes shrink and become rigid, as evidenced by the fact that the cells do not swell or undergo lysis for fairly long periods after death. The less than instantly lethal effects observed with 12 mc. per cc. of P^{32} were perhaps more comparable to the effects of lethal x-radiation delivered in amounts of 200,000 r. The reason for this is that the time required to reach a lethal level in each instance was more nearly the same. The death of the cells was observed to be accompanied by a marked change in the peripheral membrane, which rapidly lost its capacity to perform pinocytosis and to undulate in a normal active and delicate manner. The cessation of function was associated with a collapse of the membrane, leaving thin streamers attached to the cover slip. These were indications of an alteration in the adhesive quality. Unirradiated cells which show a collapse of cytoplasmic membranes due to cell death from ultraviolet irradiation, chemicals or virus infection, or, more physiologically, during mitosis, do not exhibit this phenomenon. In these instances the entire cytoplasmic membrane collapses about the cell without leaving spider web attachments to the cover slip.[8]

Many of the abnormalities associated with cell division reported here have been observed by other workers and have been documented by Stroud and Brues[9] by time-lapse motion picture analyses of x-radiation effects on living cells in tissue culture. The abnormalities of division have been considered to be due to effects on the chromatin material. It is conceivable, however, that some of the abnormalities could represent alterations in the cytoplasmic membrane. For example, in the sequence in which two daughter cells of one division almost completely separated but then fused to form a binucleated cell, the failure of complete separation of these cells might be a reflection of an increase in the adhesive character of their cytoplasmic membranes. Such an increase in adhesive-

ness might thus prevent complete cell separation at telophase. The formation of micronuclei could be related in part to alterations in the nuclear membrane and in part to fragmentation and attachment of the nucleolar material. That the nuclear membrane was significantly altered by the effects of ionizing radiations was evident from its marked infolding. How much nucleolar fragmentation and attachment contributed to the formation of micronuclei and how much damage to the nuclear membrane *per se* contributed to this process is a matter of speculation.

The converse effect of increased cytoplasmic membrane adhesiveness, namely, lessened ability of the membrane to maintain its integrity, could be found in cells exposed to 5 mc. per cc. of P^{32}. In these a marked microbubbling of the membrane took place. This resulted in the breaking off of small circular membranous fragments. This increase in cytoplasmic membrane activity was the only cellular phenomenon we observed that might be considered as representative of a stimulating effect by ionizing radiation.

The production of the multinucleated giant cells described by us and others [10] appeared to be based on two mechanisms. One was the failure of complete telophase separation of a post-mitotic cell, and the other was the fusion of cells. In our experience the latter mechanism was the more common. The cells ordinarily exhibiting this phenomenon were already binucleated or multinucleated cells. Here again we wish to emphasize that cytoplasmic fusion represented an x-radiation-induced change in the cytoplasmic membrane since unirradiated control cells rarely, if ever, displayed it. The production of mononucleated giant cells by x-radiation has been reported previously by Pomerat, Fernandes, Nakanishi and Kent [11] and by Tolmach.[12] In its simplest form this phenomenon represents growth without division. It has been considered to be due to an alteration in the apparatus necessary for cell division without a concomitant alteration in cell synthetic processes. There is some evidence to show that these enlarged cells are heteroploid, indicating that the process of DNA synthesis and the process of division are not necessarily related.

In addition to being enclosed by a membrane, the cell contains many internal membranes. In one form or another all of these membranes respond to x-radiation. The 3 most prominent membranous structures inside the cytoplasm are the mitochondria, the endoplasmic reticulum and the Golgi membranes. Ludford [13] has observed mitochondrial swelling and degeneration in cells damaged by x-radiation. In our series, more or less comparable mitochondrial alterations were noted. In the phase motion pictures and in the electron micrographs, the mitochondria were observed to form loops, to split, to develop clubbed ends and to be generally enlarged. Since the mitochondria are the site of the main energy produc-

tion of the cell, it is of interest to speculate that their damage might result in lowered energy output. This in turn could be a factor in the collapse of the peripheral membrane observed in some of the films. Some of the inclusion bodies demonstrated might have been of mitochondrial origin. The evidence for this was the multi-membranous nature of the internal structure of portions of the inclusion. The response of the endoplasmic reticulum to x-radiation was vaguely defined. The most direct evidence we offer was the inclusion body in which fragments of the endoplasmic reticulum and altered ribosomes appeared to be the main constituents. We have previously reported [14] that ribonuclease introduced into living cell cultures would induce a considerable generalized enlargement of the cell. This effect is not unlike the x-radiation effect which results in the production of unusually large mononucleated cells. It may be that these two effects have a common basis, namely, damage to the endoplasmic reticulum and its ribosomes. The effect of x-radiation on the Golgi apparatus was first described in detail by Fogg and Warren.[15] Using light microscopy, these workers pointed out that x-radiation produced an enlargement of the Golgi apparatus. Their data are in accord with our own phase contrast and electron microscopic observations. We wish to emphasize the point that the enlargement of the Golgi apparatus was in proportion to the enlargement of the entire cell and that it was an x-radiation effect expressed by a structure predominantly membranous in character.

The nuclear membrane responded to sublethal amounts of x-radiation by enlarging and infolding to form deep clefts. The characteristics of the fine structure of the nuclear membrane did not appear to be altered by sublethal amounts of x-radiation. Large or instantly lethal doses of electron irradiation caused the nuclear membrane to wrinkle and collapse slightly. These doses also resulted in a separation of the nuclear membrane from the cytoplasm so that the membrane became surrounded by a chromophobic corona when viewed with the phase microscope. Nucleolar alterations as a response to x-radiation have been reported by Duryee [16] and Warren.[17] Both of these authors have commented on the increased vacuolation of the nucleolus following x-radiation. In our own material the light microscope studies showed the nucleoli to increase in vacuolation, to enlarge, to assume bizarre shapes and frequently to have holes within a part of their structure. The ultrastructural appearance of the nucleolar responses correlated well with those observed by light microscopy. In addition they revealed a redistribution of the two main types of nucleolar granules. This particulate redistribution had some resemblance to the early stages of the nucleolar change which we have reported as a response to the carcinogens 4-nitroquinoline N-oxide and actinomy-

cin D.[18,19] Here the electron-dense particles capped the surface of the less dense particles to form a distinctive morphologic change. In our own material the nucleolar changes of mosaics and "doughnuts" represented in part a rearrangement of the two types of nucleolar granules. The dense granules clustered together and in some instances formed micro caps on the surface of the larger collections of small particles. The morphologic nucleolar changes were most probably accompanied by profound functional changes which at the moment must remain speculative. Additional studies with labeled material are in progress to elucidate some of the functional aspects of the nucleolar alterations.

Summary

The cellular and subcellular effects of two forms of ionizing radiations have been recorded by phase contrast time-lapse motion picture photography and electron microscopy. The observed functional and morphologic alterations have been correlated.

The studies indicated that ionizing radiations had profound effects on all cellular membranes. These effects could account for many of the important alterations in cell function observed during and after exposure to irradiation. As such, these observations serve to direct attention to the importance of the nongenetic manifestations of ionizing radiations.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

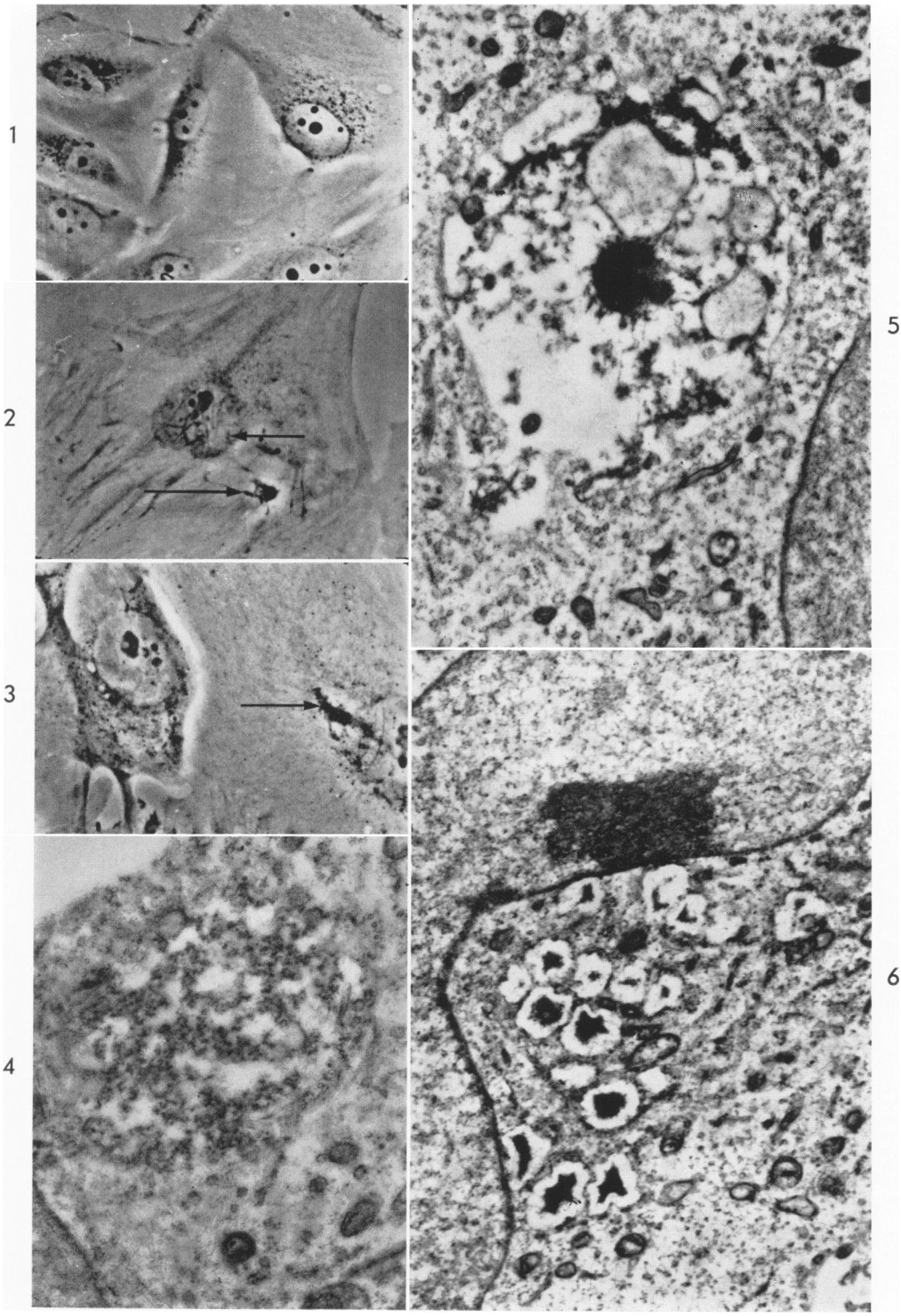
FIG. 1. Phase contrast, unirradiated Chang liver cells. The nucleoli are smooth, round and optically homogeneous. The nuclear membrane is not folded. The Golgi zone is not prominent and the mitochondria are just visible at the limits of resolution. × 21.

FIGS. 2 and 3. Phase contrast, Chang liver cells during the third week of irradiation by 0.6 mc. per cc. P^{32}. The cells are greatly enlarged and the nuclear membrane is folded. The nucleoli are enlarged, irregular in size and shape and have areas of differing optical densities. Each cell contains a cytoplasmic inclusion body indicated by the arrow. In Figure 2 the second arrow indicates deep folds and clefts in the nuclear membrane. × 21.

FIG. 4. A cytoplasmic inclusion body is manifest. Because of the small vesicles and electron-dense bodies, the inclusion is considered to be derived from the endoplasmic reticulum and its associated ribosomes. × 20,300.

FIG. 5. A membranous type of cytoplasmic inclusion is probably derived from mitochondria. × 12,000.

FIG. 6. A cytoplasmic inclusion is illustrated. The circular structures filled with lipid may represent degenerated mitochondria. × 14,500.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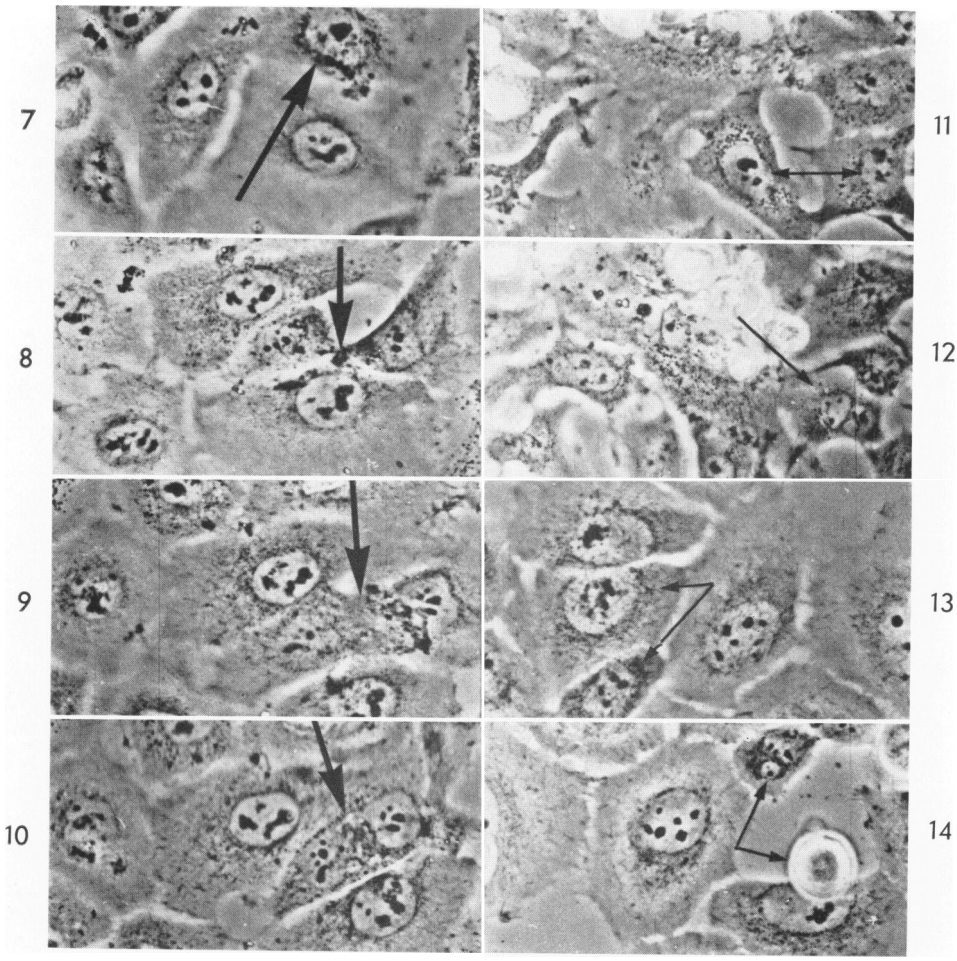
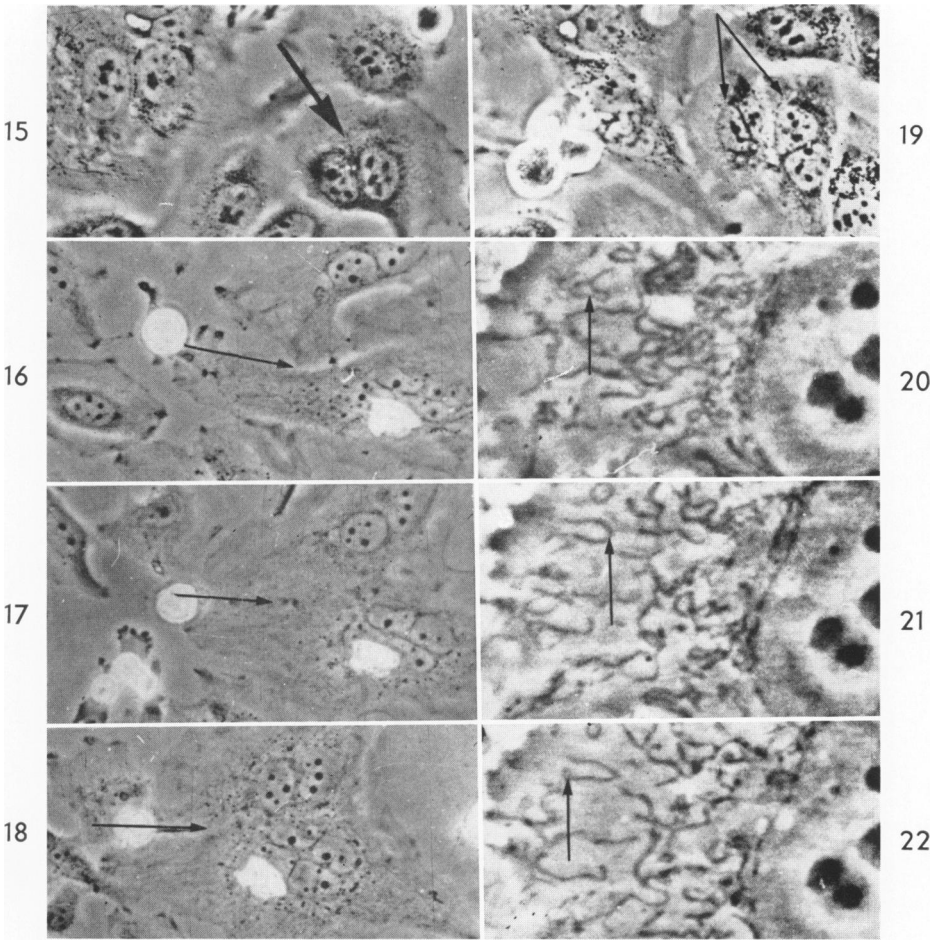


FIGS. 7 to 10. Phase contrast, telophase recombination in a liver cell after 600 r. The sequence demonstrates one method of binucleated cell production. × 21. FIG. 7. Original cell. FIG. 8. Two daughter cells at telophase. Arrow indicates a short connecting cytoplasmic bridge. FIG. 9. The telophase cytoplasmic bridge re-widens. FIG. 10. The two daughter cells recombine to form a binucleated cell.

FIGS. 11 and 12. Phase contrast, Chang liver cells after 600 r. Mitotic arrest and the formation of a binucleated cell are demonstrated. × 21. FIG. 11. Two cells prior to mitosis. FIG. 12. Cell on the left never completes mitosis, while the cell on the right gives rise to one binucleated cell.

FIGS. 13 and 14. Phase contrast, liver cells after 600 r. Binucleated cell formation, upper arrow; a cell in mitotic arrest, lower arrow. × 21. FIG. 13. Arrow indicates two cells which enter mitosis at the same time. FIG. 14. The upper cell has completed mitosis and given rise to one cell with a micronucleus in which there is a nucleolar fragment. The lower cell never completes mitosis.



FIGS. 15 and 16. Phase contrast, Chang liver cells after 600 r of x-radiation. × 21. FIG. 15. Arrow indicates a binucleated cell. FIG. 16. Arrows indicate two binucleated cells which are the result of mitosis in the binucleated cell shown in Figure 15.

FIGS. 17 to 19. Phase contrast, Chang liver cells after 600 r. The formation of a multinucleated giant cell is illustrated. × 21. FIG. 17. Arrow indicates the point of cytoplasmic contact between a binucleated cell (top) and a multinucleated cell (bottom). FIG. 18. Early stage of cytoplasmic fusion of two cells. FIG. 19. Complete cytoplasmic fusion of two cells.

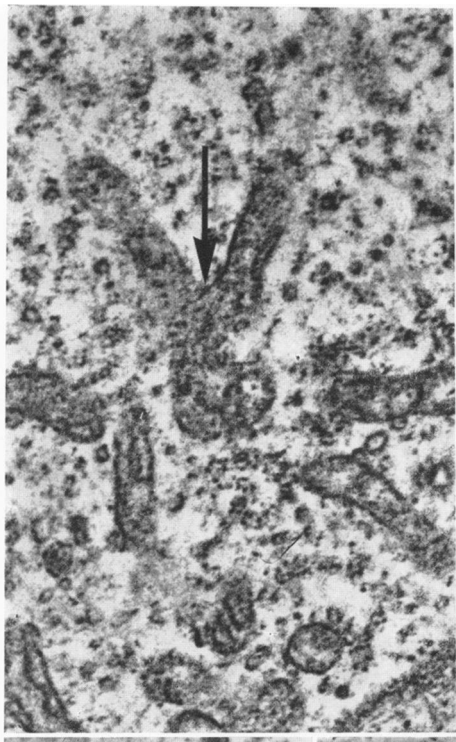
FIGS. 20 to 22. Phase contrast. Enlarged mitochondria appear in the cytoplasm of a Chang liver cell after 600 r of irradiation. × 96. FIG. 20. Arrow indicates a mitochondrion formed into a partial loop. FIG. 21. Arrow indicates the same mitochondrion after the loop has opened to form a U. FIG. 22. Arrow indicates terminal bulb-like swelling in the same mitochondrion.

FIG. 23. Arrow indicates a branched mitochondrion in a cell which had received 600 r. × 28,050.

FIGS. 24 and 25. The arrows indicate swollen and club-shaped mitochondria in cells after 600 r. FIG. 24. × 26,100. FIG. 25. × 17,400.

FIG. 26. Phase contrast, 3 weeks of exposure to 0.6 mc. per cc. of P^{32}. Arrow indicates an enlarged Golgi zone. × 21.

FIG. 27. Enlarged Golgi membranes in a cell after 600 r. × 20,300.



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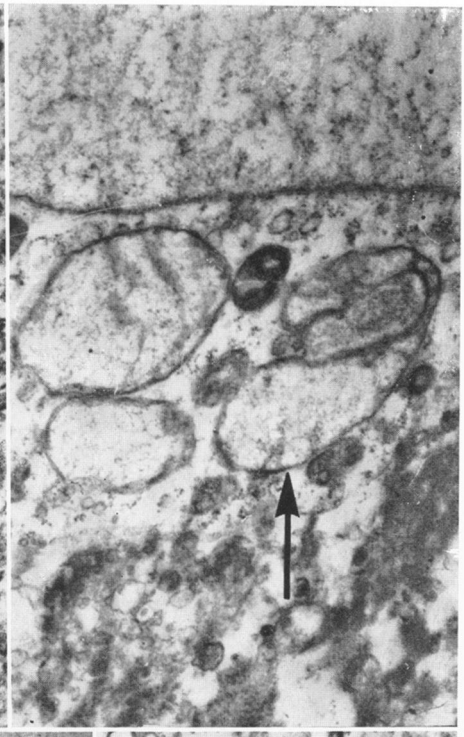

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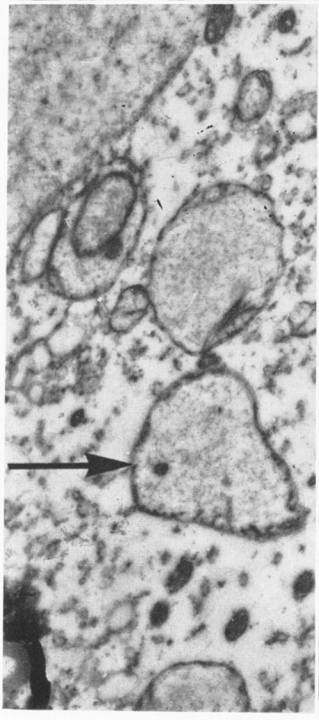

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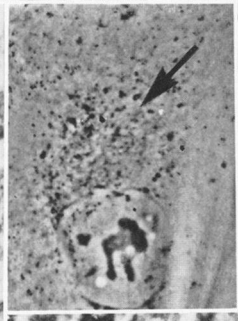

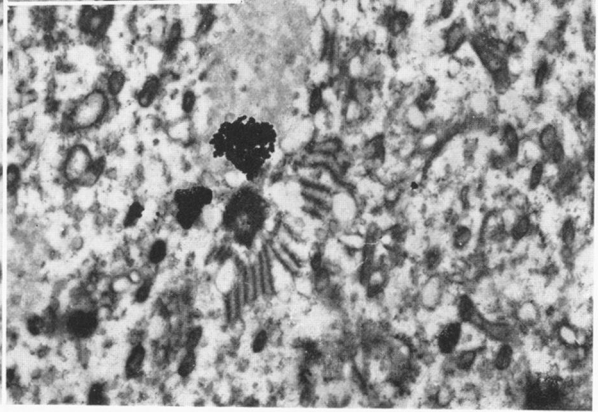

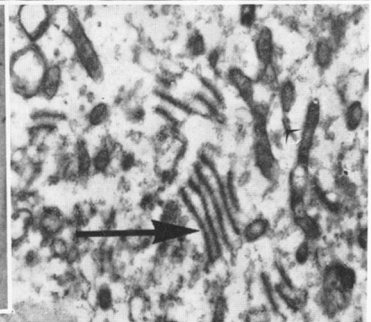

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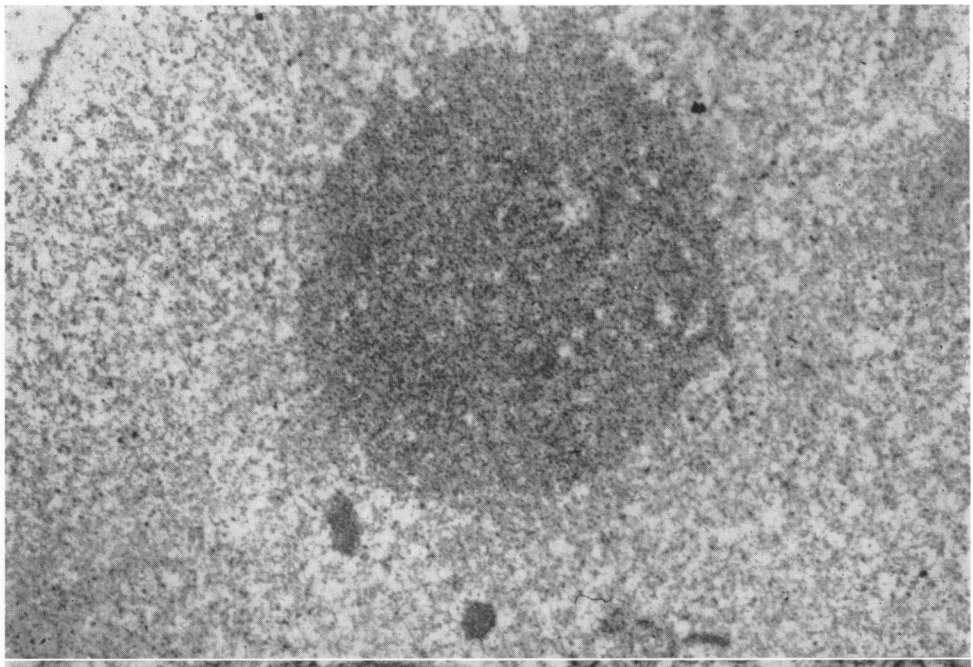

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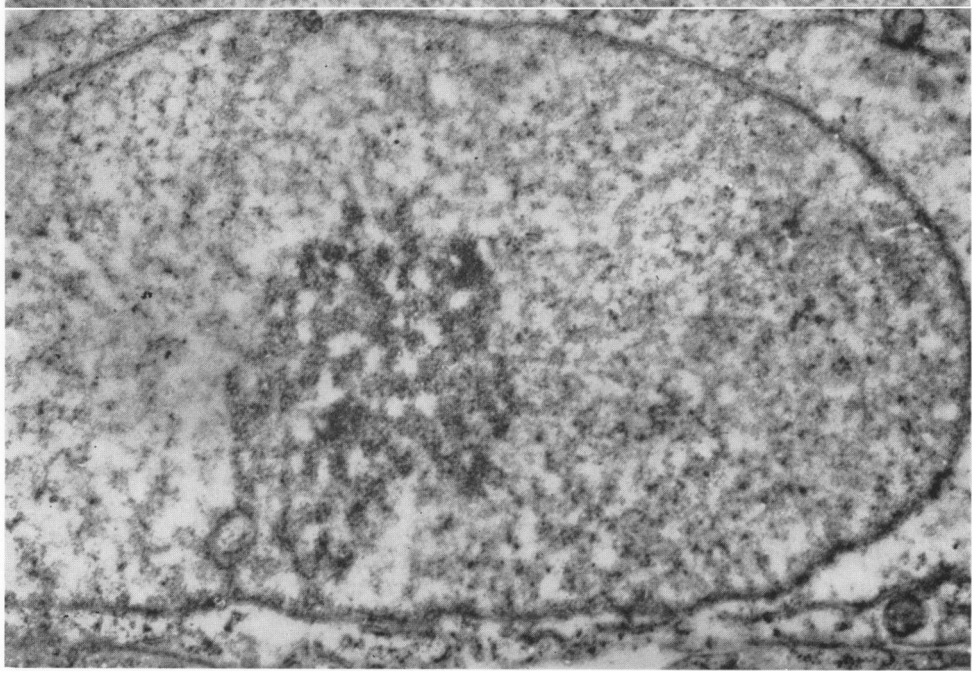

FIG. 28. Unirradiated nucleolus. This is the common arrangement of nucleolar particles. There are occasional particles with increased electron density. This nucleolus appears similar to that shown in Figure 1. × 20,300.

FIG. 29. A skein-like arrangement of the nucleolar particles contains foci of electron-dense particles. This unirradiated nucleolus would have a slightly vacuolated appearance by phase microscopy. × 20,300.

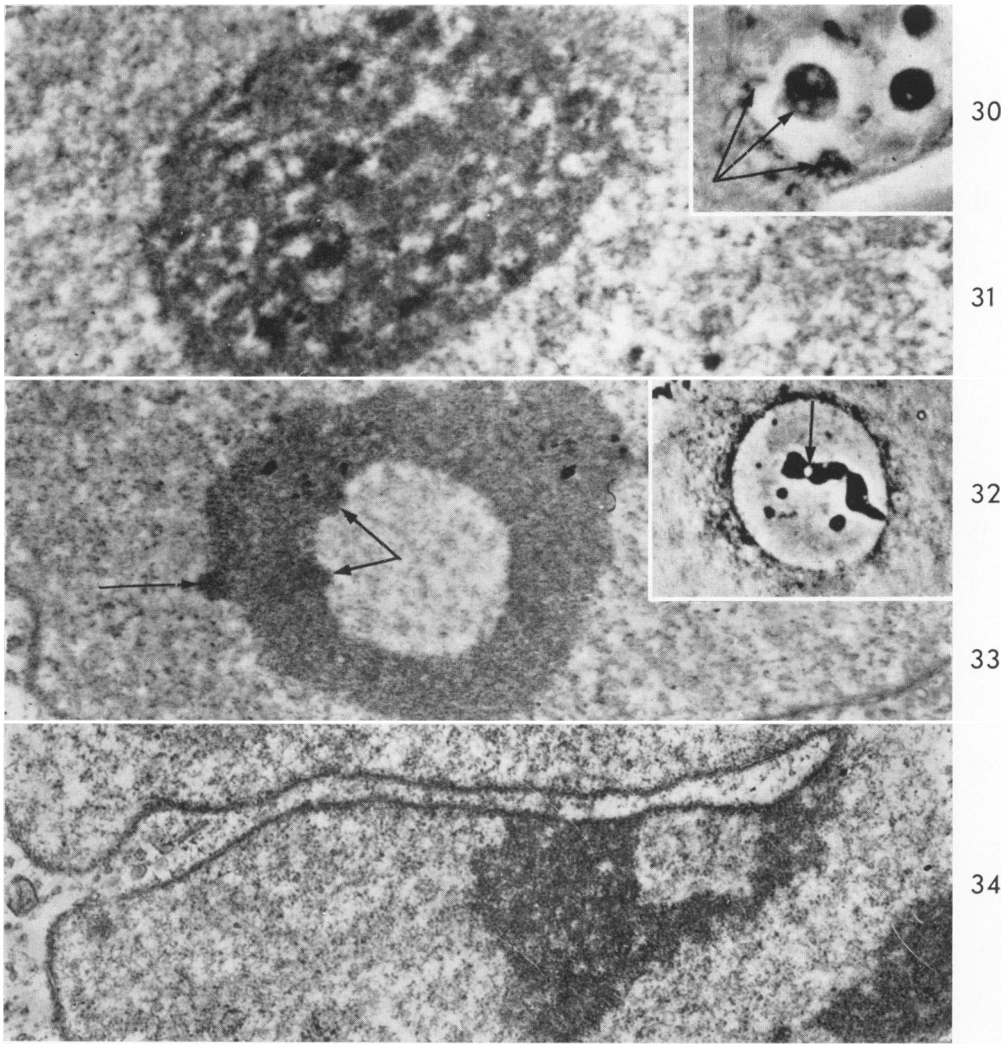

FIG. 30. Phase contrast, liver cell nucleus after 600 r. The central arrow indicates a vacuolated nucleolus. The side arrows indicate folds in the nuclear membrane. × 96.

FIG. 31. Nucleolus of cell after 600 r. The nucleolus has a mosaic pattern and a more distinct condensation of the electron-dense particles. × 20,300.

FIG. 32. Phase contrast, cell several days after exposure to 600 r. The entire cell is greatly enlarged. The arrow indicates a hole in the nucleolus. × 21.

FIG. 33. Nucleolus of a cell after 600 r. The nucleolus has a central hole. The arrows indicate projecting buds of electron-dense nucleolar particles. × 16,500.

FIG. 34. Nuclear membrane of a cell after 600 r. The nucleolus is attached to a deep fold in the nuclear membrane. × 23,200.

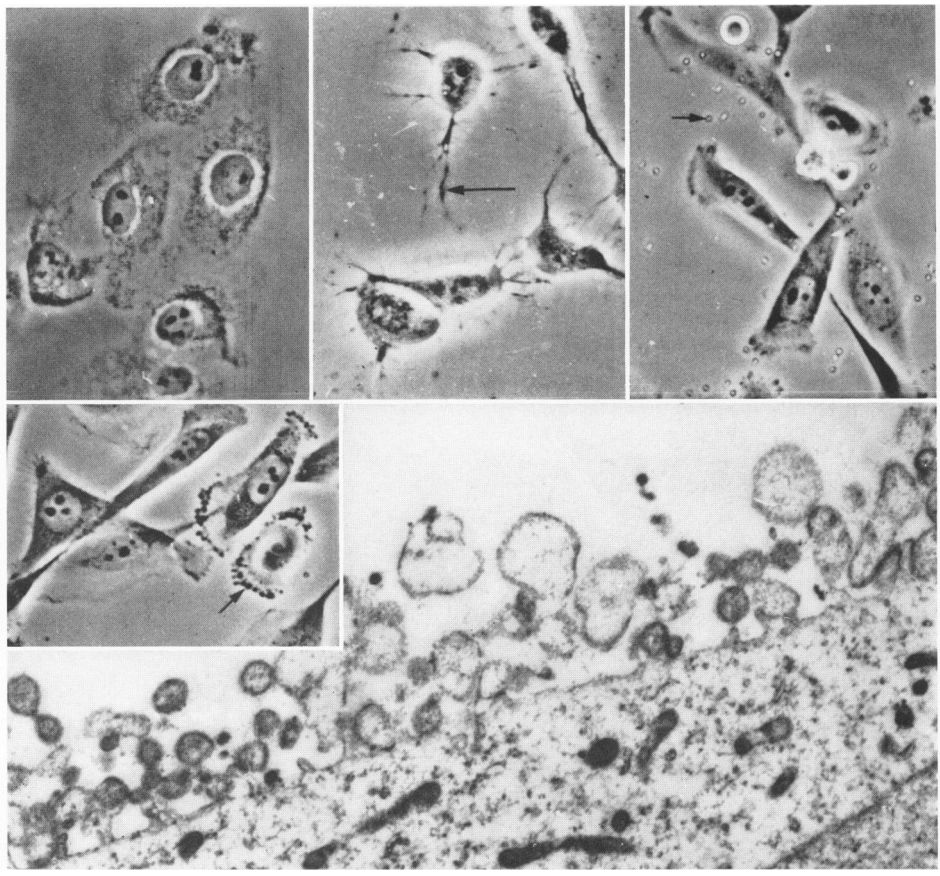

FIG. 35. Phase contrast, liver cells after irradiation by 18 mc. per cc. of P^{32}. The cells appear fixed with the nuclei slightly collapsed, wrinkled and surrounded by a chromophobic corona. The cytoplasm is moderately contracted. × 21.

FIG. 36. Phase contrast, a field of liver cells after the incorporation of 12 mc. per cc. of P^{32} into the media. After several hours the cells collapse. Arrow indicates an attached cytoplasmic streamer with entrapped lipoprotein droplet. × 21.

FIGS. 37 and 38. Phase contrast, cells during exposure to 5 mc. per cc. of P^{32}. × 21. FIG. 37. Arrow indicates cell with an intense micro-bubbling of the peripheral membrane. FIG. 38. Arrow indicates small detached circular fragments of the cytoplasmic membrane.

FIG. 39. The cytoplasmic border of a liver cell after 600 r. Numerous cytoplasmic membrane bubbles appear at the surface. The appearance is similar to that shown in Figure 37. Some of the bubbles contain small vesicles and particles felt to be endoplasmic reticulum. Some of the larger membrane fragments within the bubbles may be portions of mitochondria. × 14,500.