3
Cellular Responses

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THIS paper is concerned with some aspects of the organization of cells, particularly as seen with the light microscope, and the results of some experiments on dividing cells in tissue culture as shown by time-lapse motion pictures. The paper is illustrated by portions of a few key frames from the motion pictures.

There have been several important periods in the study of cellular organization. One of them was the period of descriptive histology, embryology, and cellular pathology of the nineteenth century. In it a variety of important processes in the development and function of cells were portrayed largely, but not exclusively, on the basis of dead material. This was followed by a period of extreme skepticism in which a number of investigators claimed that nearly everything seen after fixation and staining was artifact; they believed that only what could be seen in the living cell existed in it. There were even claims that nuclei could not be seen in living cells and hence were artifact. Actually, nuclei are easily demonstrable during life. This period of skepticism was helpful in some respects but it also did great harm because some of its claims were too destructively critical. One of the rewards of living in the present period is to see that many of the so-called artifacts of the previous century have been shown to exist in living cells by our more modern methods of investigation. I remember distinctly the first time I saw a living cell with the newly developed phase-contrast microscope. What I saw was like an iron-hematoxylin stained cell moving around under the microscope and offering complete verification, at the light-microscope level, of the existence of certain structures previously known mainly in preparations of dead cells.

As our methods of morphologic, chemical, and physical analysis become more powerful and as the study of the minute structure of living systems reaches ever closer to the molecular level, it becomes more and more obvious that the old problem of what is artifact in our observational material is still with us.

I shall show a series of newt cells cultured in diluted chicken plasma. In the time-lapse movies, constituents of a typical cell are shown as it goes through the process of cell division (mitosis). In the film, the reality becomes apparent of some structures that to some of you may have been only words. Three cells show the effects of either ionizing or ultraviolet, total cell irradiation. The succeeding five dividing cells demonstrate some of the effects of localized irradiation of small parts of them. In the cells which were irradiated throughout their extent, several abnormalities appear, while in those irradiated locally, the number of abnormalities is much smaller. The comparison of local *versus* total cell irradiation gives a little insight into some aspects of the machinery of cell division, a problem which Raymond Z rkle and the author began to work on some eight or nine years ago with the aid of our proton microbeam.[1] A few years later, Robert Uretz began to work with us in our study of mitosis; the simple ultraviolet microbeam, which he devised, has been of great help.[2,3] With it, one can aim directly at a structure and watch it as it is being irradiated.[4,5] Others in our laboratory, especially E. W. Taylor and R. P. Perry, have also contributed to our knowledge of what happens in dividing cells. Zirkle[6] has recently reviewed the methods and results of microbeam irradiation which started with the work of Tschachotin with ultraviolet light in 1912.[7]

In our proton microbeam, the protons start with an energy of 2.0 Mev but lose perhaps a quarter of this as they pass through two layers of mica, each 5 μ thick. One of these seals the delivery opening of the Van de Graaff generator; the other is the cover slip for the culture. Although there is a small percentage of scatter, if the culture is essentially in contact with the microaperture, 85 to 90% of the protons bombard an area about 2.5 μ in diameter, and practically all of the protons pass within a circle of 8 to 10 μ diameter at the target. (The indicated variations reflect slight differences in approximation of the mica cover slip to the microaperture.) The scatter is not important with small numbers, but obviously becomes serious with hundreds of thousands. With a more powerful generator, it would have been possible to deliver a single proton into a target of 1 μ^2, but the smallest number we have been able to deliver with our generator is a burst of about 20.

The Uretz microbeam makes use of a reflecting objective which brings all wavelengths of visible and ultraviolet light into approximately the same plane. This device has an important advantage over the proton microbeam in that the size and shape of the area to be irradiated can be varied easily from circles 2 μ in diameter to much larger ones, or to slits, or to irregular shapes of various dimensions. We can also compare the effects of microbeams of monochromatic and heterochromatic ultraviolet light with those of the proton microbeam.

The machinery by which a plant or animal cell becomes two cells is probably much the same in all, although much more is known about the process in nucleated cells than in bacteria. Much of what is shown here has been known in the descriptive sense since about 1880 in the work of Strassburger and especially of Flemming, among others. But we know very little more today than these early workers knew about the causal

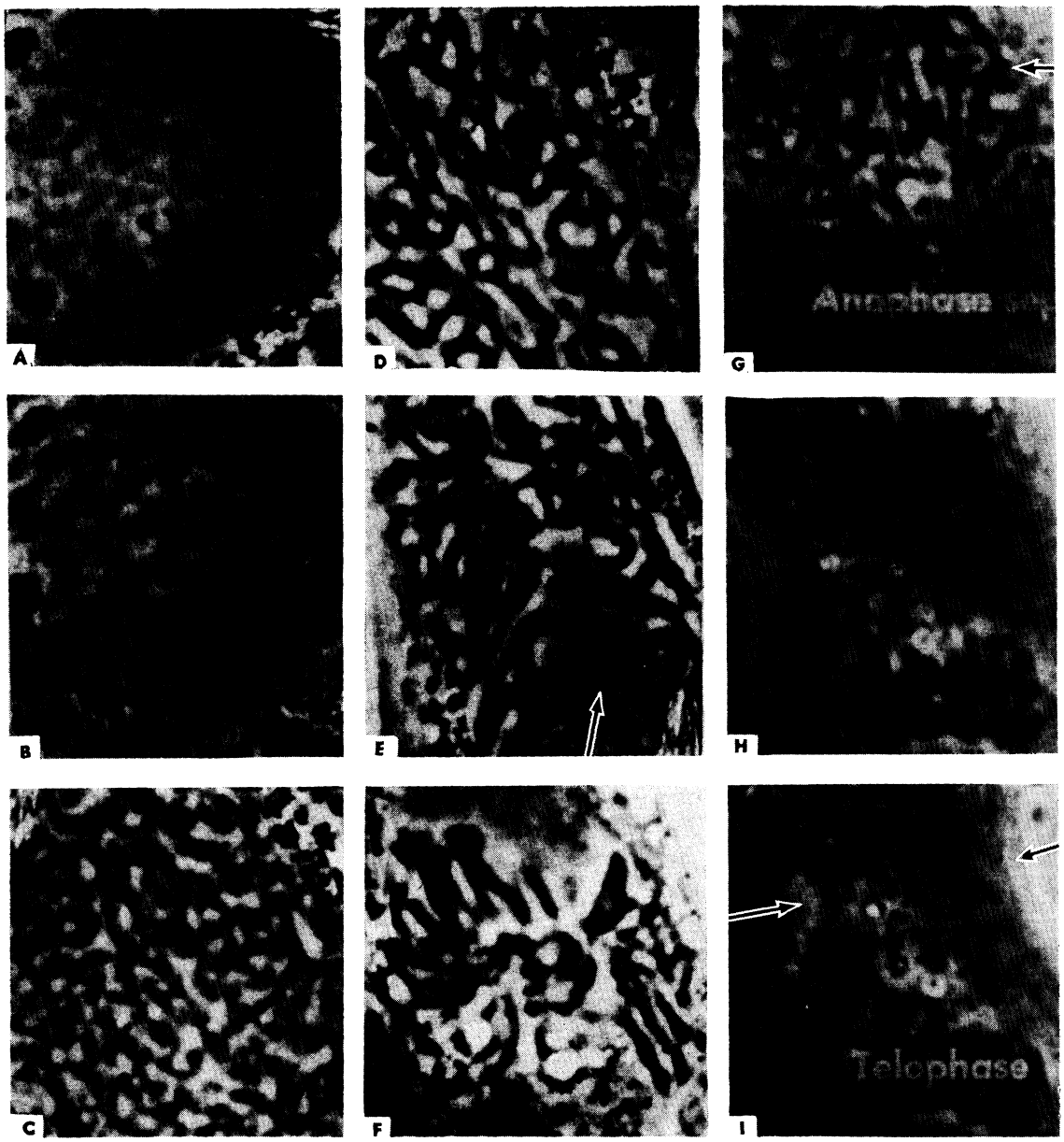


FIG. 1. This sequence [(A) to (I)] shows some of the main stages in mitosis. The nucleus at (A) is in a very early stage in the change of a relatively nongranular nucleus into chromosomes. The two large bodies are nucleoli. At (B), the cell has moved slightly and three nucleoli are visible. The nuclear granules begin to form vague cords which are much more definite at (C) and especially so at (D) where they have become typical chromosomes. (E) shows the change in arrangement of chromosomes from prophase into metaphase at (F). In (G), each chromosome is dividing into two daughter chromosomes (as at arrow) which are beginning to move to opposite poles of the cell (anaphase). At (H), the chromosomes have completely separated and in (I) reconstruction of the daughter nucleoli is beginning and the cell body is constricting (arrows). The arrow in (E) points to a centriole.

mechanisms involved in this complicated, meticulous process by which the two daughter cells inherit equal amounts of genetic material.

In the films, the area of the cells shown is approximately 40 by 60 μ, and the motion pictures are speeded up about 100 times. When present, the clock indicates the lapse of time. Figures 1 to 9 are from the time-lapse motion picture shown at the presentation of the paper, photographed with medium dark, phase-contrast microscopy; 1400×. All cells are from newt heart explanted in a mixture of one part chicken plasma and nine parts amphibian Tyrode solution. The division process in these cells at 70°F may take as long as 6 or 7 hr or as little as 4 hr. These differences, at the same temperature, are unexplained.

UNTREATED CELL IN MITOSIS

In cell 1, a typical mitosis, one can see tiny fat droplets surrounding the nuclear membrane, the filamentous and granular mitochondria, the nucleus with large nucleoli, and numerous, irregular small granules [Fig. 1(A)]. The author used to question whether the movement of granules in the cytoplasm was Brownian motion, since one sees it only in the speeded-up time-lapse movies, and not by direct observation of the living cells. But Taylor studied this phenomenon and convinced him that it was Brownian motion.

As the film continues, the small granules in the nucleus become more numerous and darker and gradually form long snake-like bodies—the chromosomes [Figs. 1(A)–1(D)]. They are about 2 μ thick and some 30 μ or more long. The nucleoli disappear suddenly and we do not see what happened to them [between Figs. 1(B) and 1(D)]. The nuclear membrane also disappears and about the time that it does the chromosomes begin a continuous, irregular movement. Warren Lewis, who has spent many years studying living cells, called this the "Dance of the Chromosomes."

The cell contains two other important small structures called centrioles, one above and another below the group of chromosomes. The poles of the spindle are connected with the centrioles. The area with practically no granules is part of the developing spindle [Fig. 1(E)]. After some time, a pale stripe appears within each chromosome and extends through its length. Suddenly, the chromosomes divide along this stripe and the daughter chromosomes (or chromatids), each a half of an original chromosome, move apart [Fig. 1(G)] and form the two separate daughter nuclei [Figs. 1(H) and 1(I)]. Then the cell body constricts, the whole mass separating into two new cells, each with a nucleus. The temporary excrescences on the surfaces of the cell are normal when they are not more extensive than in this cell. In the irradiated cells, which are seen next, they may be much larger, more numerous, more violent in movement, and have longer persistence.

The process of cell division goes on in our bodies hundreds of millions of times a day in order to replace those cells which are normally lost for various reasons. In the vast majority of cases, these mitoses are executed as meticulously as in this cell.

RESPONSES TO TOTAL CELL IRRADIATION

The next three cells show a number of different phenomena as a result of total cell irradiation. Cell 2 received 200 r of 200-kv x-rays. The film starts with the cell in an early stage of mitosis. As a consequence of the irradiation, an abnormality develops instead of the orderly cell division which occurred in the untreated cell. Chromosomes start to separate but the separation is not complete [Fig. 2(B)]. Then cell constriction occurs about the mass of chromosomes [Fig. 2(C)] and forms a dumbbell-shaped nucleus connected by a long bridge of chromosomal material extending between the two daughter cells [Fig. 2(D)].

Cell 3 received 350 r of 200-kv x-rays. Instead of producing a relatively simple malformation, as produced by 200 r in the previous cell, several abnormalities occur. The chromosomes make an abortive attempt to separate into their daughter chromosomes [Fig. 3(A)], violent "bubbling" appears on the cell surface, and two constrictions of cytoplasm occur [Fig. 3(B)]. We have seen bubbling go on for days in cytoplasm pinched off as in this case. Nuclear material now separates into two irregularly sized groups [Fig. 3(C)], but at one point there is a tiny connection between them, and the smaller group of chromosomes spurts over into the larger one, making a bi-lobed nucleus.

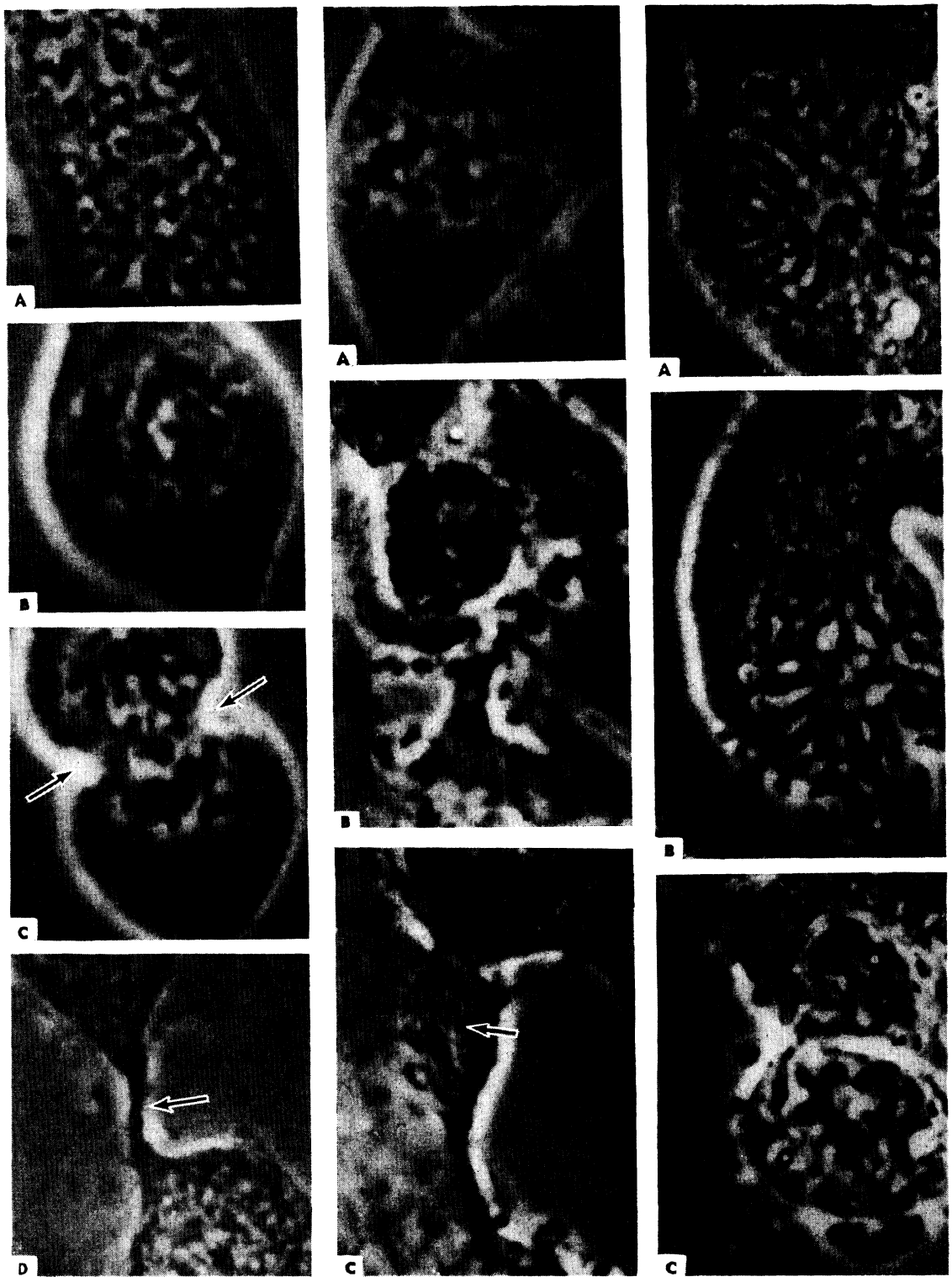
Cell 4 received ultraviolet light over its whole body. Despite the marked abnormalities which result, the cell will eventually give rise to daughter cells which are able to exist for a time at least in tissue culture. Immediately after being irradiated, some of the motion of structures within the cell ceases, but some of it gradually returns. For a short period, there is an irregular arrangement of chromosomes and then terrific bubbling movements begin to appear [Fig. 4(B)]. The chromosomes and cytoplasmic granules move in and out of the bubbles. We call this "type 4 bubbling," the most extreme degree. The term "bubbles" is a misnomer as they are protuberances of cytoplasm and not bubbles; their nature is completely unknown. Through it all, the chromosomes stick together, more or less. The bubbling motion gradually begins to subside. Then the chromosomes separate into one large and one small group which move apart and a constriction of the cytoplasm develops [Fig. 4(C)]. As nuclear reconstruction begins, it becomes apparent that the two nuclear masses are connected by a thin chromosomal bridge.

RESPONSES TO PARTIAL CELL IRRADIATION

In the next 5 cells, very small areas of nucleus or cytoplasm were irradiated by protons or ultraviolet light.

There is a widely held view in the radiobiological literature that chromosomes are damaged secondarily as a result of irradiation of the cytoplasm. The experiments on the next two cells clearly refute this claim for the newt cells in our culture. In cell 5, the area indicated by cross-hairs [Fig. 5(A)], about 14 μ from the nearest chromosomes (arrows), was irradiated with 28 300 protons. The intensity of irradiation of this bit of the cell is enormous when one realizes that each proton equals 600 rep. Nevertheless, the resulting mitosis is perfectly normal [Figs. 5(B) and 5(C)].

Cell 6 received 60 protons localized over a small area of two chromosomes [see cross-hairs and arrowhead in Fig. 6(A)]. The daughter chromosomes on the nonirradiated side separate cleanly, while those on the irradiated side are stuck together. The daughter chromo-



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somes move apart as a group, much as in an opening hinge pinioned on the irradiated parts of the chromosomes [Fig. 5(B)]. This produces a temporary chromosomal bridge [Fig. 6(C)]. The difference between the failure of the 28 300 protons delivered to cytoplasm to produce any visible change in the chromosomes of the previous cell is in sharp contrast to the effect of 60 protons delivered locally to chromosomes in this cell.

Areas 8 μ in diameter of the next three cells were irradiated with ultraviolet light. The first of these cells (number 7) is in early mitosis (prophase). The spindle has not yet formed and, as a result of cytoplasmic irradiation, will not form. There is an irregular arrangement of chromosomes characteristic of this stage. After the localized, cytoplasmic, ultraviolet irradiation, the irregular configuration persists [Fig. 7(B)]. The chromosomes shift around from time to time and there are pulsations and temporary extrusions of the cell surface. After several hours, a regrouping of the chromosomes occurs suddenly. They do not give rise by longitudinal splitting to a double number of daughter chromosomes, as in normal mitosis. Instead, haphazard numbers of *whole* chromosomes aggregate into two groups which separate one from another [Fig. 7(C), arrows]. Then the cytoplasm constricts and two daughter cells are formed. Such cells obviously differ completely in their genetic complements from each other and from those cells arising from a normal mitosis.

In the next cell (number 8), it is possible to follow the aggregation of the two groups of chromosomes more easily than in the previous one wherein cytoplasmic irradiation had prevented the appearance of the spindle. In this cell, which is in the mid-phase of mitosis, the spindle is fully developed. The area to be irradiated is shown at the cross-hairs [Fig. 8(A)]. After irradiation of a small part of the cytoplasm (8 μ) with ultraviolet light, the spindle breaks down and the chromosomes lose their characteristic, butterfly-like arrangement, becoming irregularly disposed in the center of the cell [Figs. 8(B) and 10]. A pale longitudinal line becomes visible in each chromosome and marks the usual line of cleavage of each chromosome into its two daughter chromosomes (chromatids). The pale line disappears, however, before the whole chromosomes aggregate into two irregular groups [Fig. 8(C), arrows]. In cells so treated, the number of chromosomes in each new nucleus may vary from 9 to 15 or even more, instead of 22 which is characteristic of this species. Constriction occurs and the daughter cells move apart.

Since, in the experiments on cells 7 and 8 (and nearly 100 others similarly treated), irregularly sized groups of whole chromosomes move apart in the absence of the spindle, it would seem that the spindle serves primarily as a mechanism by which *daughter chromosomes* are guided as they are moved apart by unknown forces.

From the days of Flemming on, there have been many theories about the machinery of mitosis. Some of the models, such as those based on rubber bands, have some resemblance to what is seen in some stages of mitosis, but they contribute nothing to an understanding of the forces determining the processes in mitosis. It has been claimed that the daughter chromosomes become separated by electrostatic forces or by a localized imbibition of water, or that the spindle is made of a contractile material which pulls them apart. Although cognizant of many claims to the contrary, it is probably fair to say that there has been no delineation at all of the nature or amount of the forces involved in mitosis.

Nor do we have any idea of the nature of the force which moves the irregular groups of whole chromosomes apart in the absence of the spindle. It is known that, in the early stages of mitosis, the two centrioles separate and take position at the apices of the spindle, and that there is a temporary attraction between the centrioles and the kinetochores of some of the chromosomes. Then, this attraction ceases and all the chromosomes move into a position midway between the two centrioles (metaphase). In the next stage of division (anaphase), the daughter chromosomes separate from their mates and move with their kinetochores foremost toward the centrioles. In those cells in which the spindle is abolished, we assume that, during the separation of the groups of whole chromosomes, those chromosomes close to each of the centrioles go with it to a daughter cell.

The last cell (number 9) shows a different phenomenon after localized irradiation (8 μ in diameter) of chromosomes with ultraviolet light [Fig. 9(A)]. A dramatic change in the refractive index of the chromosomes appears in the irradiated area and produces what we call a "pale" spot [Fig. 9(B), arrows]. This change does not appear if much more than half of the chromosome mass is irradiated. However, irradiation of as little as 2 μ of a chromosome will produce it. The mass of chromosomes immediately around the pale area becomes sticky and cannot separate into chromatids, while those some distance away can. From this there results an irregular, lobated single nucleus with malformed chromosomes and a pale spot in the center [Fig. 9(C)].

In fixed preparations, the pale area also appears pale with most of the usual stains. A similar appearance

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FIG. 4. This cell received a 2.5-min exposure of a germicidal ultraviolet lamp 2.5 cm from the culture. At the time of irradiation, the cell showed typical metaphase configuration. At (A), the chromosomes show an irregular rosette appearance. A few minutes later, the cell begins to undergo violent bubbling and at (B) the chromosomal mass is being extruded into one of the bubbles. Later, there are multiple constrictions (C).

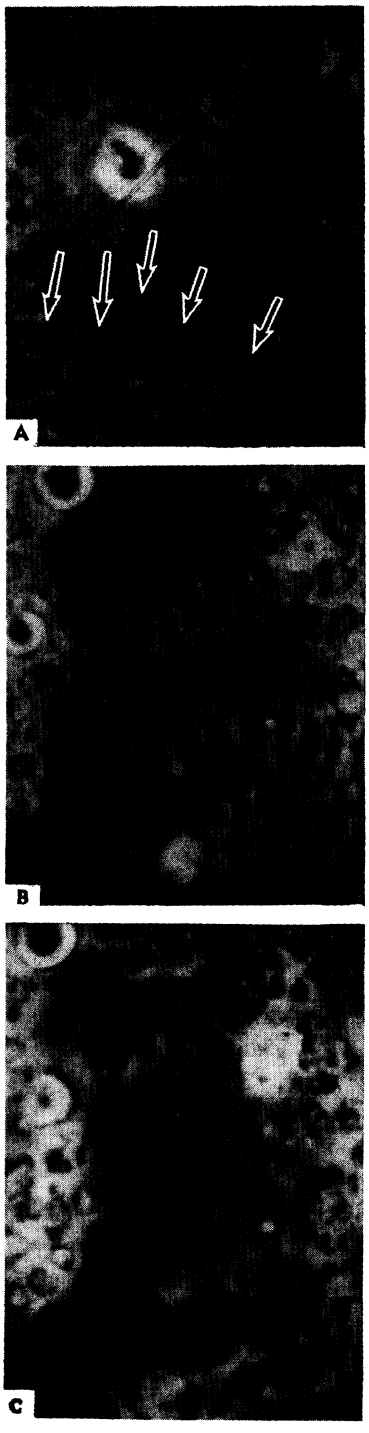

FIG. 5.

FIG. 6.

FIGS. 5 AND 6 show the difference in effect of localized irradiation of cytoplasm *versus* localized irradiation of chromosomes in dividing cells. The cell shown in Fig. 5 received 28 300 protons at the area indicated by the double cross-hairs. The nearest chromosomes are indicated by the arrows. This cell divided into two by a perfectly normal mitosis as shown in (B) and (C). Figure 6 shows a totally different picture as a result of irradiating small parts of two chromosomes (indicated by cross-hairs and arrow) with 60 protons during metaphase. As shown in Fig. 6(B), the chromosomes separated normally into daughter chromatids except those at the point of irradiation where, as indicated by the arrow, the chromosomes are stuck together. As a result, the two daughter nuclei (N,N) are joined by a chromosomal bridge indicated by the three arrows.

results from the application of the Feulgen stain for deoxyribonucleic acid.[8] This suggests that, in the development of the pale spot, the nucleic-acid moiety of the irradiated part of the chromosomes may have been so altered that it does not respond to the stain or has actually left the area. Ultraviolet-absorption studies of the pale spot by Perry show a diminished absorption at 260 mμ (Fig. 11).[8]

Zirkle has spent much time in the past few years determining the relative effectiveness of the following wavelengths (225, 240, 250, 260, 270, 280, and 300 mμ) on paling. He found the action spectrum to have the

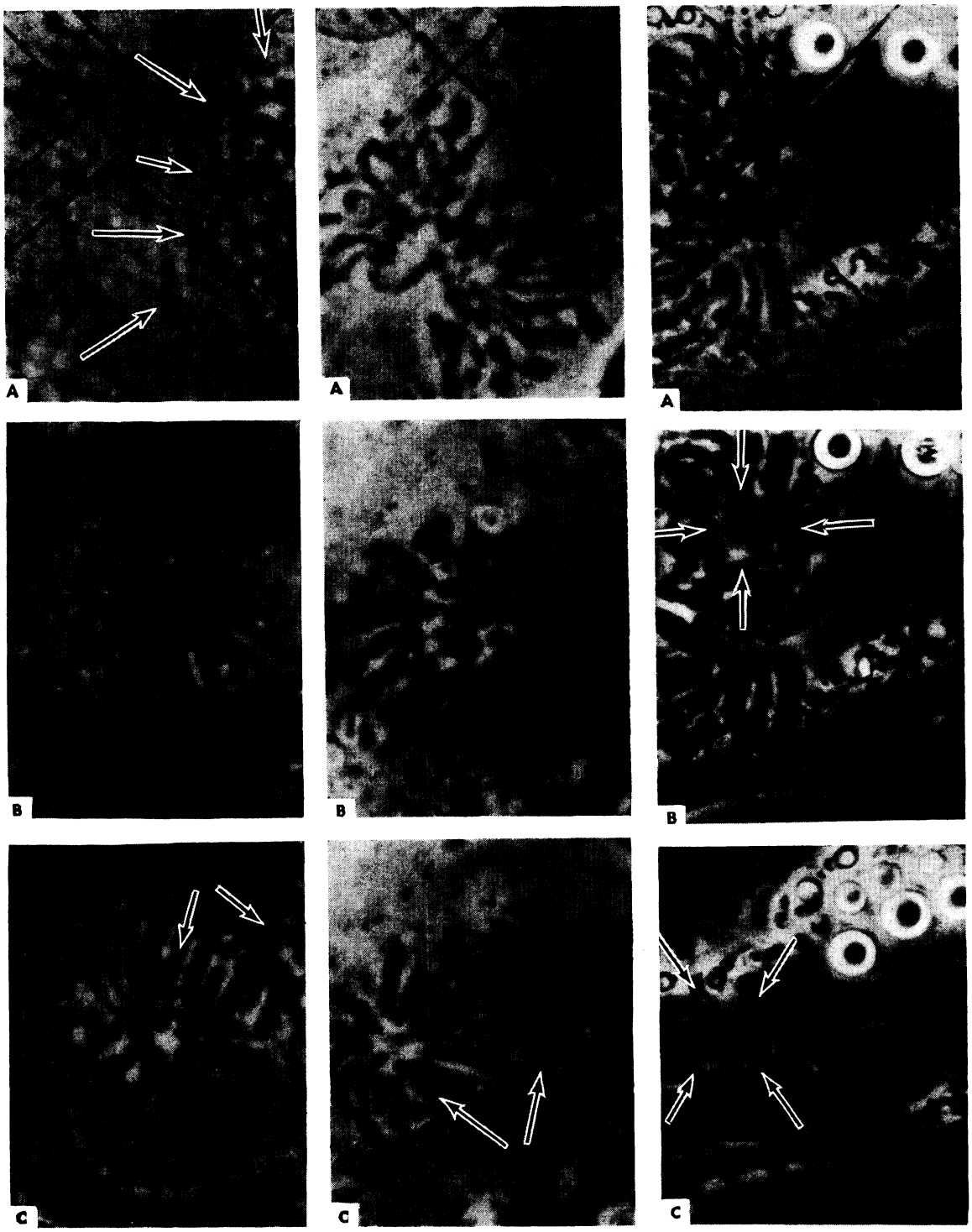

FIG. 7. FIG. 8. FIG. 9.

FIG. 7. This cell in prophase received localized, ultraviolet irradiation at the area indicated by the cross-hairs. The nearest chromosomes are shown by the arrows. The spindle did not form and the chromosomes occupied an irregular clumped area (B) and later separated as whole chromosomes into two unequal groups, indicated by the arrows in (C).

FIG. 8. The cell, in metaphase with a clearly marked spindle, received localized, ultraviolet irradiation of the area indicated by the cross-hairs. The spindle disappeared promptly and the chromosomes show a haphazard clumping (B) much like that in 7(B). In cell 8, as in the previous one, whole chromosomes separate into two daughter clumps, shown by the arrows in (C).

FIG. 9. This cell, in metaphase, received ultraviolet, localized irradiation of an area of chromosomes 8 μ in diameter, indicated by the cross-hairs. Within a minute, the chromosomes showed a typical "paling" reaction (B). The cell attempted to divide but was unable to execute a complete anaphase as the chromosomes immediately around the pale area were very sticky. There resulted a single irregularly lobed nucleus with a paled spot (arrows) in (C).

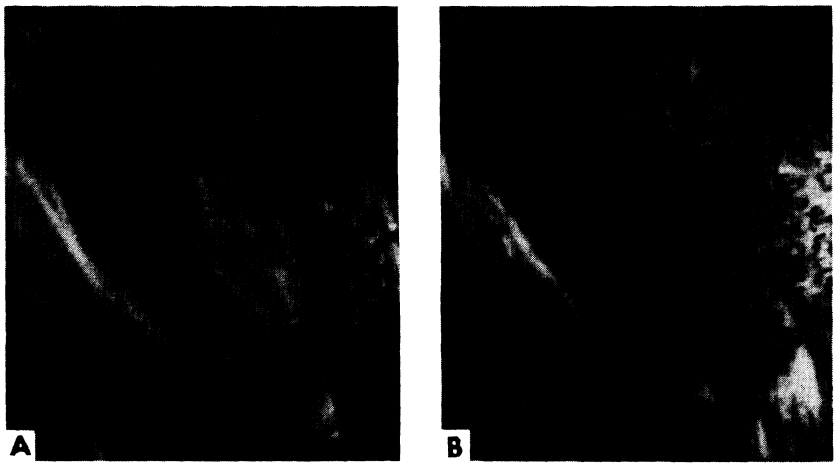

FIG. 10. Newt cell in metaphase as seen with polarized light. (A) The spindle is prominent before irradiation. (B) The spindle has disappeared shortly after localized, ultraviolet irradiation of cytoplasm. Courtesy R. B. Uretz.

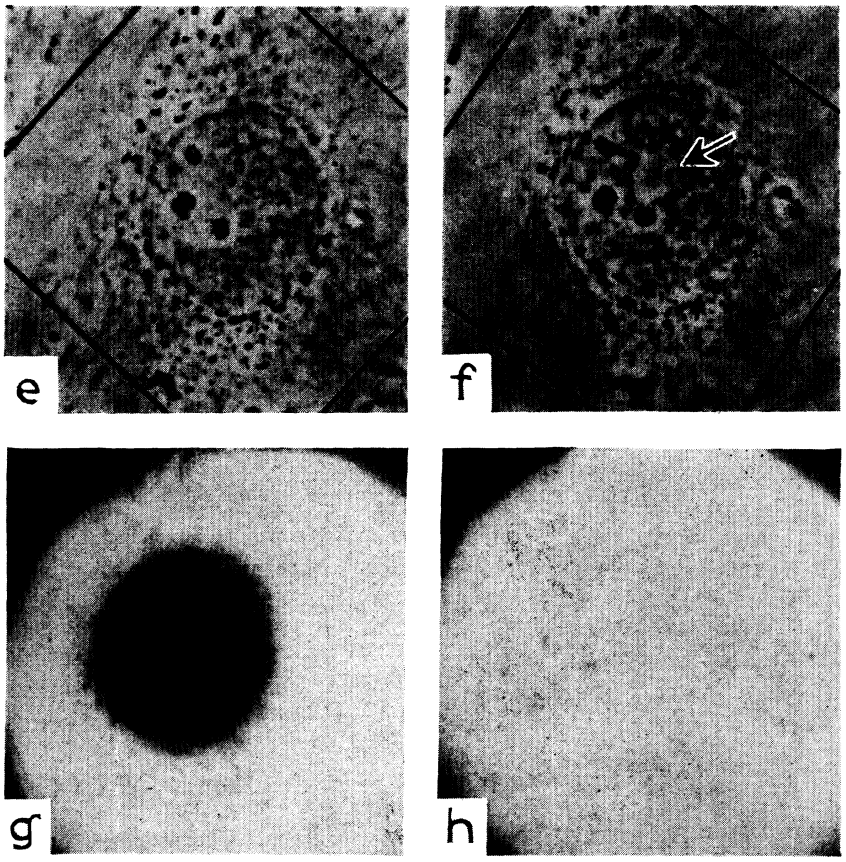

FIG. 11. Newt cell in prophase. (e) Before irradiation; (f) shortly after ultraviolet irradiation of 4-μ diam circle of nucleus. The paled area at the place of irradiation is shown by the arrow. (g) After irradiation, photographed by 260 mμ light, and (h) photographed by 310 mμ light. The diminished absorption of the paled area at 260 mμ is obvious (after R. P. Perry[8]).

general absorption spectrum typically shown by proteins containing aromatic amino acids. There is a minimum at 250 mμ and a broad maximum between 260 and 280 mμ.

The relative effectiveness of the two types of irradiation is roughly as follows: Irradiation with ultraviolet light of an 8-μ spot of a chromosome will require 5 sec to make it sticky and about 10 sec to make it pale, while spindle destruction will need about 150 sec of irradiation of an 8-μ spot of cytoplasm. Chromosomes irradiated locally with 2 Mev protons become sticky with 20 protons and pale with tens of thousands. It seems to require a million or more protons delivered to a small area of cytoplasm to destroy the spindle; we have not done many such experiments and this is a very approximate figure.

SUMMARY

(1) Irradiation of dividing newt cells in culture with 200-kv x-rays or with ultraviolet light produces few or many abnormalities depending on the amount of radiation applied.

(2) Localized irradiation of chromosomes with a few tens of protons makes them sticky. But no abnormalities in the mitotic process result when a small area of cytoplasm is irradiated with 28 300 protons.

(3) Localized irradiation of cytoplasm with ultraviolet light prevents formation of the spindle or destroys it if it has already formed. In such cells, groups of whole chromosomes (not chromatids) move apart, usually in inequal numbers, to form the daughter nuclei of the dividing cell.

(4) The spindle thus seems to provide a mechanism for guiding the chromatids in anaphase, since, in the absence of a spindle, groups of whole chromosomes can move apart and form the nuclei for the daughter cells.

(5) Localized ultraviolet irradiation of chromosomes changes the index of refraction at the affected area. Such pale spots stain very faintly for DNA with the Feulgen method and absorb very little at 260 mμ.

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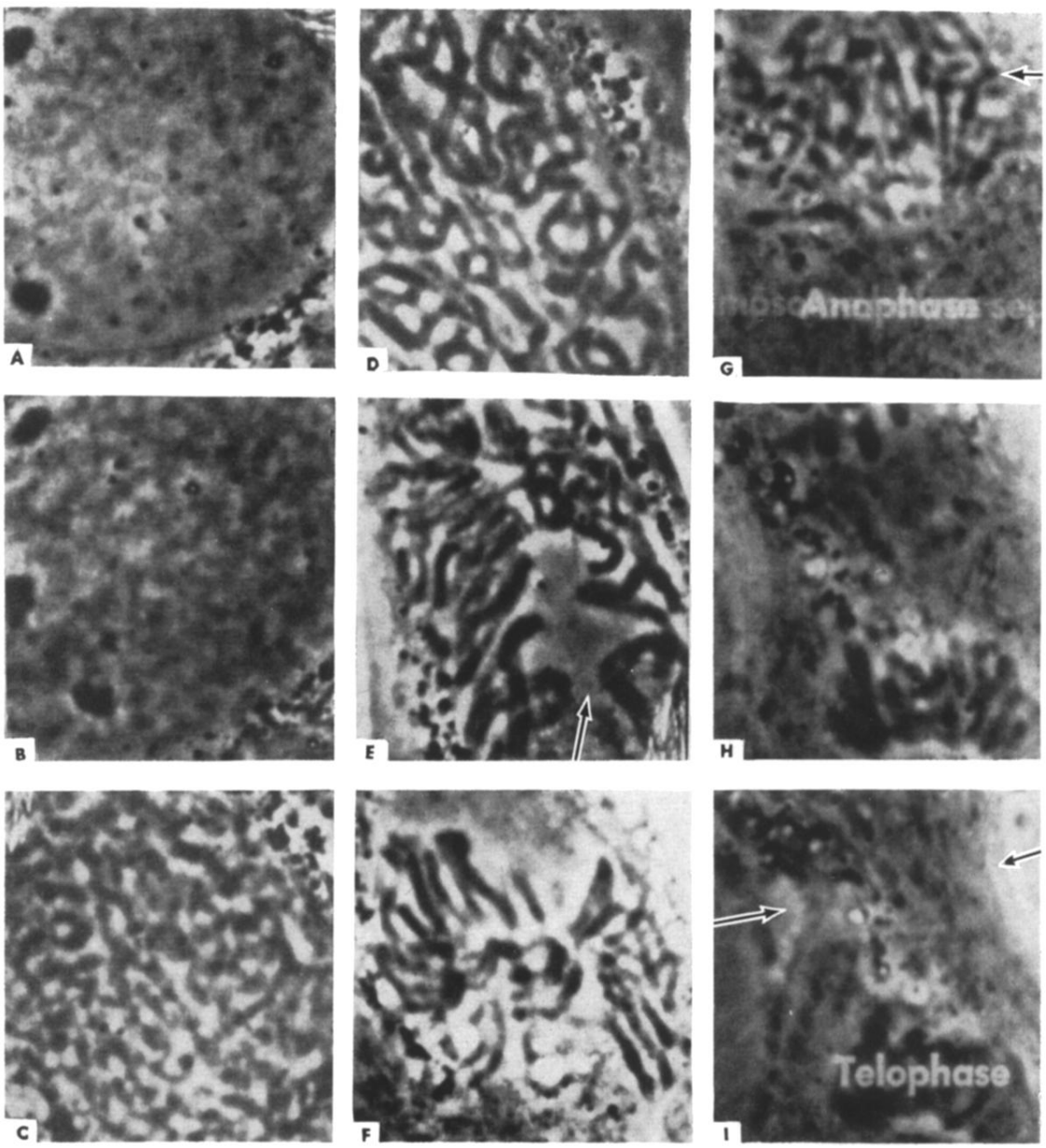

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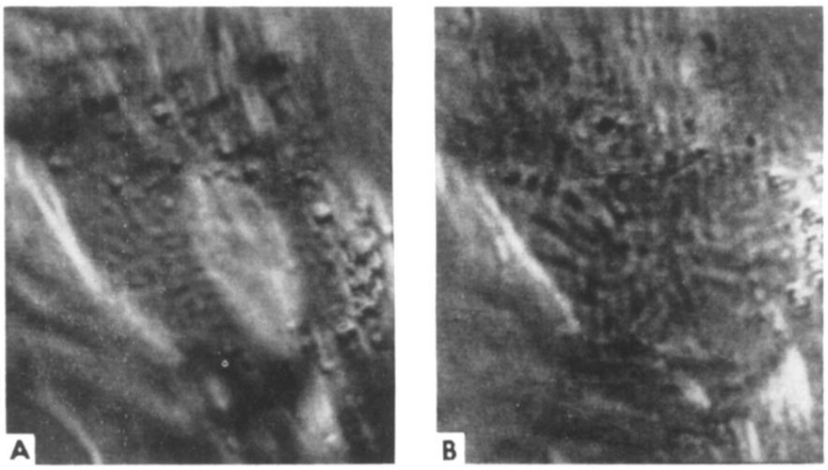

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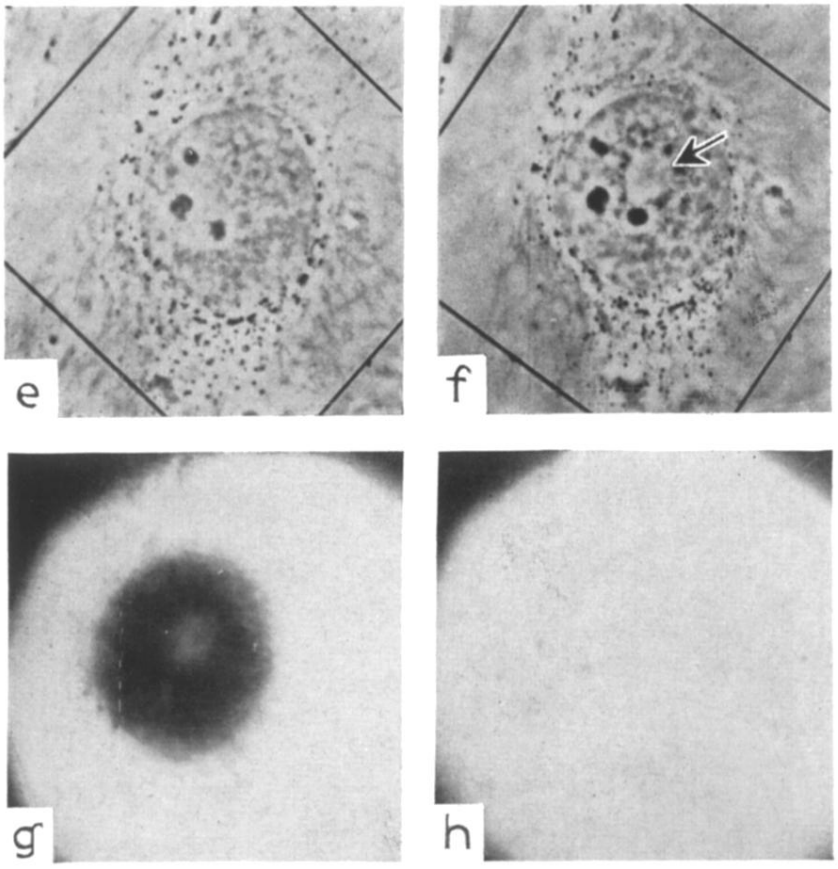

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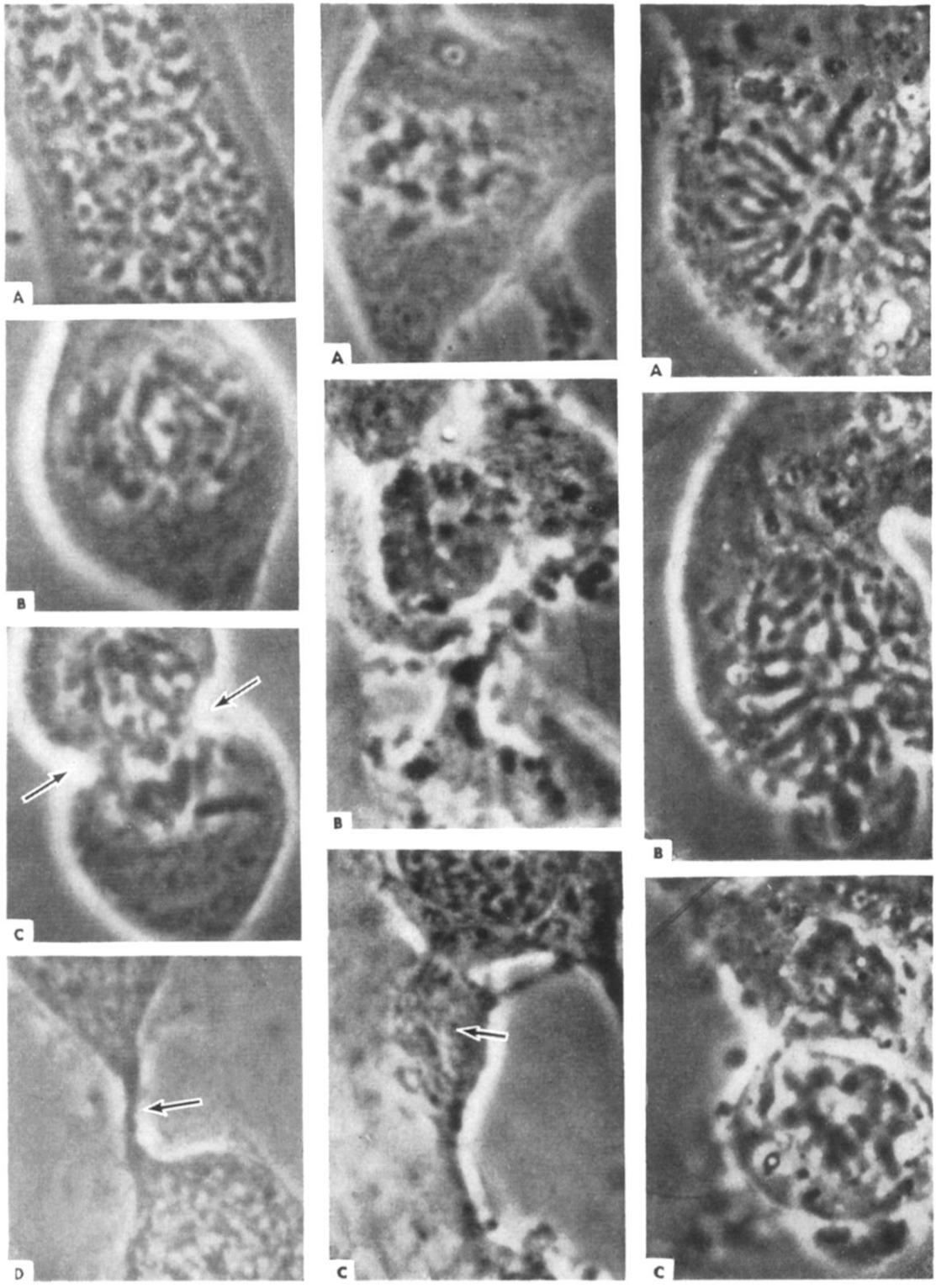

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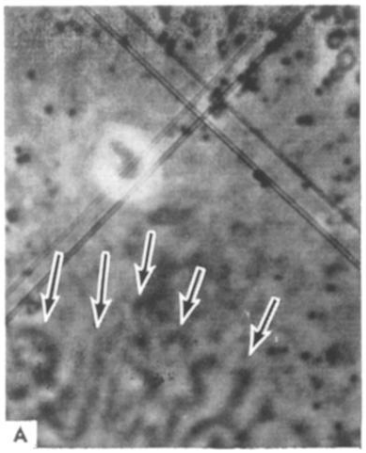
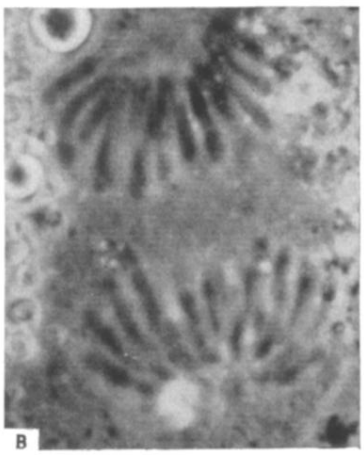




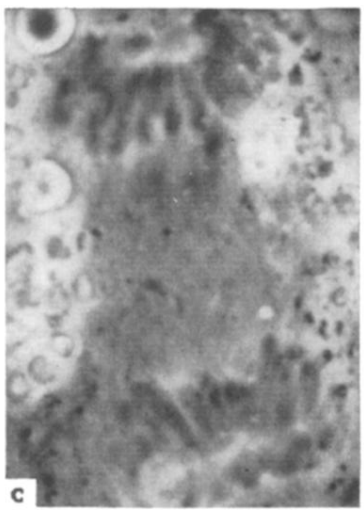
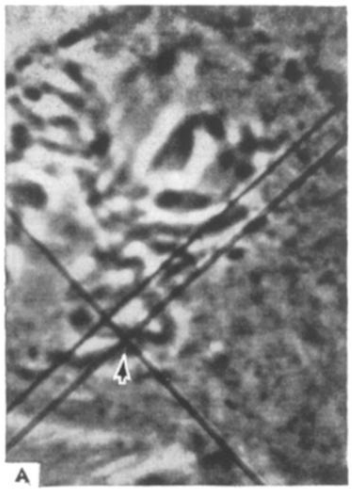
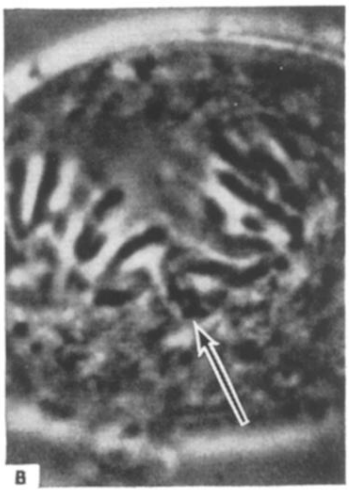


FIG. 5.





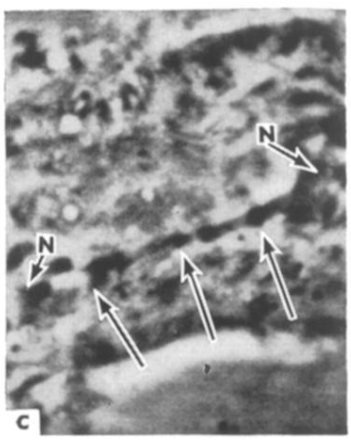


FIG. 6.

FIGS. 5 AND 6 show the difference in effect of localized irradiation of cytoplasm *versus* localized irradiation of chromosomes in dividing cells. The cell shown in Fig. 5 received 28 300 protons at the area indicated by the double cross-hairs. The nearest chromosomes are indicated by the arrows. This cell divided into two by a perfectly normal mitosis as shown in (B) and (C). Figure 6 shows a totally different picture as a result of irradiating small parts of two chromosomes (indicated by cross-hairs and arrow) with 60 protons during metaphase. As shown in Fig. 6(B), the chromosomes separated normally into daughter chromatids except those at the point of irradiation where, as indicated by the arrow, the chromosomes are stuck together. As a result, the two daughter nuclei (N,N) are joined by a chromosomal bridge indicated by the three arrows.

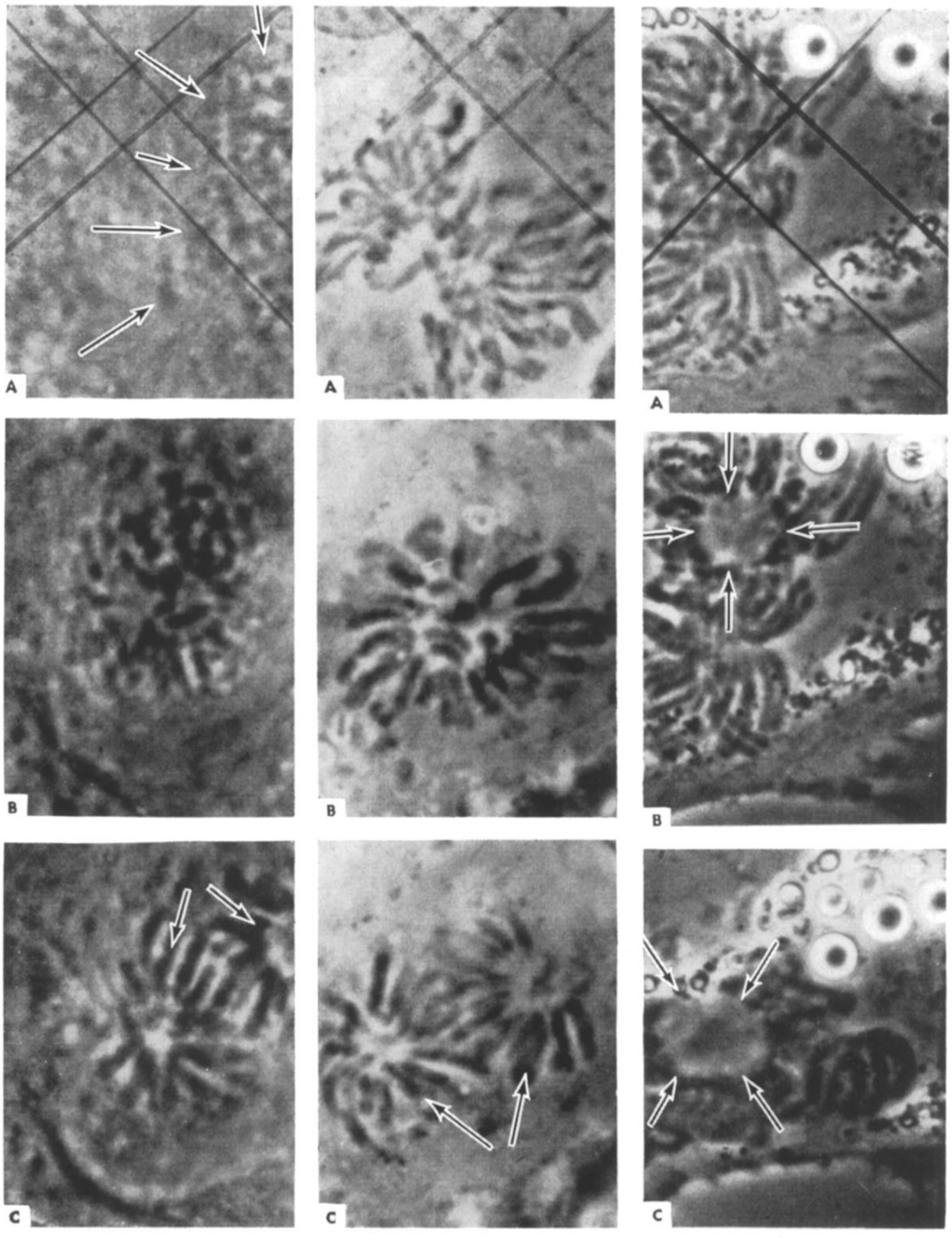


FIG. 7.

FIG. 8.

FIG. 9.

FIG. 7. This cell in prophase received localized, ultraviolet irradiation at the area indicated by the cross-hairs. The nearest chromosomes are shown by the arrows. The spindle did not form and the chromosomes occupied an irregular clumped area (B) and later separated as whole chromosomes into two unequal groups, indicated by the arrows in (C).

FIG. 8. The cell, in metaphase with a clearly marked spindle, received localized, ultraviolet irradiation of the area indicated by the cross-hairs. The spindle disappeared promptly and the chromosomes show a haphazard clumping (B) much like that in 7(B). In cell 8, as in the previous one, whole chromosomes separate into two daughter clumps, shown by the arrows in (C).

FIG. 9. This cell, in metaphase, received ultraviolet, localized irradiation of an area of chromosomes 8 μ in diameter, indicated by the cross-hairs. Within a minute, the chromosomes showed a typical "paling" reaction (B). The cell attempted to divide but was unable to execute a complete anaphase as the chromosomes immediately around the pale area were very sticky. There resulted a single irregularly lobed nucleus with a paled spot (arrows) in (C).