

INDUCTION OF PROPHASE IN INTERPHASE NUCLEI BY FUSION WITH METAPHASE CELLS

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ABSTRACT

Fusion of an interphase cell with a metaphase cell results in profound changes in the interphase chromatin that have been called "chromosome pulverization" or "premature chromosome condensation." In addition to the usual light microscopy, the nature of the changes has been investigated in the present study with electron microscopy and biochemical techniques. Metaphase and interphase cells were mixed and fused at 37°C by means of ultraviolet-inactivated Sendai virus. After cell fusion, morphological changes in interphase nuclei occurred only in binucleate cells which contained one intact set of metaphase chromosomes. Irrespective of the nuclear stage at the time of cell fusion, the morphologic changes that occurred 5–20 min later simulated very closely a sequence of events that characterizes the normal G₂-prophase transition. Radioautography revealed that, late in the process, substantial amounts of RNA and probably protein were transferred from the interphase nucleus into the cytoplasm of fused cells. Thus, the findings indicate the existence in metaphase cells of factor(s) which are capable of initiating biochemical and morphological events in interphase nuclei intrinsic to the normal mitotic process.

INTRODUCTION

Fusion of an interphase cell with a metaphase cell by means of infectious or UV-inactivated Sendai virus results in profound structural alterations of the interphase nucleus (1, 2, 10, 12–18, 32, 33, 35). The phenomenon has been termed (22) "chromosome pulverization" (CP), because of an extremely fragmented appearance of the chromatin. The nuclear envelope concomitantly undergoes extensive disruption (1, 32). Because these changes in the interphase nucleus depend on fusion with a metaphase cell, we (17, 18, 33) and Johnson and Rao (14) have proposed that normal cellular factors in the metaphase cell, which are needed for mitosis in that cell, cause the morphology of the interphase nucleus to undergo these changes.

We have obtained support for this hypothesis in

demonstrating (18) that protein synthesis late in the G₂ period of Chinese hamster cells DON before exposure to the virus is required if the metaphase cell is to affect the interphase nucleus after fusion. Additionally, it was observed that micronuclei produced by thymidine-³H treatment and out of mitotic phase with the main nucleus of the cell but untreated with Sendai virus (or any other virus to our knowledge) also underwent CP (13).

Even though the factor(s) responsible for CP are very probably normal cellular and not viral components, the question as to whether the change termed CP, and called "premature chromatin condensation" (PCC) by Johnson and Rao (14), in DON cells is the end-result of a sequence of normal morphological changes induced by

such factors must be considered separately. This paper presents for the first time certain light microscope findings strongly indicating that the changes in CP resemble normal prophase of mononucleate cells. Furthermore, this conclusion is supported by new electron microscope and biochemical evidence.

MATERIALS AND METHODS

Cells and Virus

A Chinese hamster cell line DON, a cell stock of the American Type Cell Culture Collection, Rockville, Md., was grown at 37°C either in monolayer or in suspension in RPMI 1630 medium (21) supplemented with 10% fetal calf serum, containing 100 µg/ml of penicillin and streptomycin. A strain of Sendai virus (23) concentrated at 20,000 hemagglutinating units (HAU)/ml of glucose-free Hanks' solution was freshly prepared in a manner described before (15), and inactivated by ultraviolet light.

Cell Synchrony

Metaphase cells were obtained by mitotic arrest with Colcemid, as described by Stubblefield and Klevecz (34). A log phase culture in monolayer was exposed to Colcemid at a concentration of 0.06 µg/ml for 3 hr at 37°C. Metaphase cells were detached by shaking and passed through four layers of cheese cloth, and washed three times with ice-cold medium. Each T-60 flask received about 10^7 cells, and the incubation of cells in monolayer was initiated at 37°C. From time to time, the cells were pulsed for 10 min with 1 µCi/ml of thymidine- ^3H , and radioautography was performed as previously described (17). 95% of the cells were in the G_1 phase 15 hr, and 85% in the S phase 7 hr, after release from the mitotic arrest (Fig. 1). Purity of the cells in G_2 was about 70% (Fig. 1). Such cultures were the source of G_1 , S, and G_2 cells, which were fused with metaphase cells prepared separately.

Cell Fusion and Observations

Metaphase and interphase cells, 5×10^6 each, were suspended in a total of 1.0 ml of medium containing 2000 HAU of inactivated Sendai virus and 0.06 µg of Colcemid.

After the virus adsorption (23) at 1°C for 15 min, the suspension was transferred to an incubator maintained at 37°C. After the cell fusion had reached a plateau (15), the suspension was diluted with 4 ml of prewarmed medium containing Colcemid and reincubated. At subsequent time intervals, samples of the cell suspension were chilled to 1°C. The cells were either treated with hypotonic 15 mM sodium

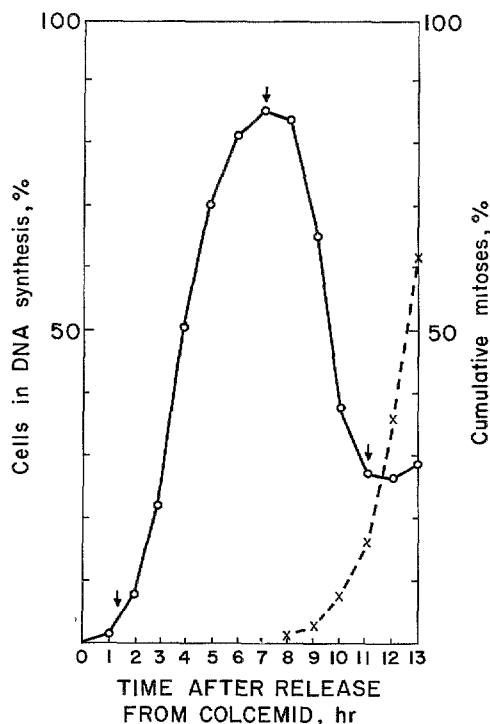


FIGURE 1 DNA synthesis in synchronized DON cells. At definite time intervals after metaphase, the fraction of cells engaged in DNA synthesis was examined by ^3TdR pulse labeling (○—○). Cumulative mitoses at any time point represent the summation of mitotic cells found at that time point with those found at the previous time points (x—x). The arrows from left to right indicate sampling of G_1 , S, and G_2 cells, respectively.

citrate, pH 6.5, and then fixed (17), or fixed directly, without the hypotonic treatment, after centrifugation. The fixative was acetic acid-methanol, 1:3. After changing the fixative three times, the cells were spread on slides and air-dried. Cells were stained with Giemsa's.

Radioautography

In order to examine the release of nuclear RNA and proteins, radioautographic techniques were used. Interphase cells in log phase were exposed for 30 min to 10 µCi/ml of uridine- ^3H (6.7 µCi/µmole), arginine- ^3H (45 µCi/µmole), lysine- ^3H (7.0 µCi/µmole), or leucine- ^3H (56 µCi/µmole). After subsequent trypsinization, they were washed three times with ice-cold fresh medium, fused with metaphase cells, and harvested and fixed as described above. The slides were coated with Kodak nuclear track emulsion NTB 2, exposed for 7 days at 4°C.

processed in Kodak D-19 developer for 2 min, and stained with Giemsa's.

Electron Microscopy

For electron microscope examinations, the medium containing the cells incubated with virus for 10–45 min was centrifuged at 600 *g* for 5 min, and the pellets were fixed in 3% glutaraldehyde buffered with Millonig's phosphate buffer (19) at pH 7.3. After 30 min the cells were rinsed with the buffer and postfixed for 60 min at 4°C in 1% osmium tetroxide in the buffer. The pellets were rapidly dehydrated with graded concentrations of ethanol and then embedded in Epon-Araldite mixtures according to the technique of Mollenhauer (20). Control cells in medium lacking Colcemid and virus were prepared in the same manner. Thin sections were cut on an LKB ultratome and stained in 2% uranyl acetate followed by lead citrate according to Reynolds (29). Observations were made with a JEM-7 electron microscope (Jeolco U. S. A. Inc., Medford, Mass.). Micrographs were taken at original magnifications of 5,000–15,000 and then enlarged photographically.

Prophase-Like Alterations in Interphase Nuclei after Fusion with Metaphase Cells

For convenience, binucleate cells which resulted from fusion and contained an interphase nucleus and a metaphase set of chromosomes will be called I-M cells. Such cells amounted to approximately 17.5% of the binucleate cell population. Cell fusion was completed within 10 min after addition of the virus.

Observations of the events immediately after fusion could not be made if hypotonic treatment was employed (12–18) before fixation. In fact, the processes occurring up to 20 min after fusion could be observed only in directly fixed cells. The I-M cells were observed at time intervals up to 20 min after fusion was complete. Representative morphology of such cells fixed directly is shown in Figs. 2–5. At 10 min after fusion 64% of the I-M cells contained interphase nuclei in which prophase had been induced. No noticeable changes in the interphase nucleus had occurred when fusion was complete, except for slightly enhanced heterochromatic regions (Fig. 2). During the next 5 min after fusion conspicuous condensation of chromatin began in the interphase nuclei, and chromatin networks were formed (Fig. 3); no visible changes in metaphase chromosomes of I-M cells occurred. At this time,

nuclear envelopes and nucleoli still appeared to persist. 10–15 min after fusion the interphase nuclei in the I-M cells were characterized by thick chromatin networks with one or two nucleoli centering in them (Figs. 4 and 5); at these times the chromatin of the interphase nuclei was unfragmented and closely resembled the chromatin in normal prophase of the control cells (compare with Figs. 6 and 7). At this stage there was complete disruption of the nuclear envelopes, which could be observed with light microscopy.

Thus, the process observed in I-M cells within 10–15 min after fusion stimulated normal G₂-prophase transition.

The appearance of the I-M cells at 20 min after fusion is shown in Figs. 8 and 9, and these may represent more advanced stages of the induction of prophase. At this time, 82% of the I-M cells exhibited interphase nuclei with this morphology.

Electron Microscope Studies

UNTREATED CELLS: Untreated cells were used to investigate morphologic changes occurring at the early stage of mitosis under our culture conditions, with particular emphasis on the state of the chromatin. As shown in Fig. 10, prophase was characterized by the condensation of chromatin inside the nuclear envelope. The nuclear envelope clearly consisted of a double membrane with nuclear pores. Simultaneously, the nucleolus in prophase underwent a distinguishable morphological transition from the interphase nucleolus, as has been described by other workers (3, 4, 7, 30).

FUSED CELLS CONSISTING OF INTERPHASE NUCLEI AND METAPHASE CHROMOSOMES: Log phase cells were exposed to Colcemid for 3 hr and fused in the same manner as described above. Fused cells, which included both interphase nuclei and metaphase chromosomes, were examined at various periods of time after fusion. Fig. 11 is representative of such cells.

The metaphase chromosomes appeared intact. The interphase nucleus resembled that of an untreated cell in prophase. The condensed chromatin was aligned just inside the nuclear envelope and along the nucleolus and was more electron-opaque than the chromatin of metaphase chromosomes. The nucleolus showed uniformly dispersed granular elements. Polysomes were

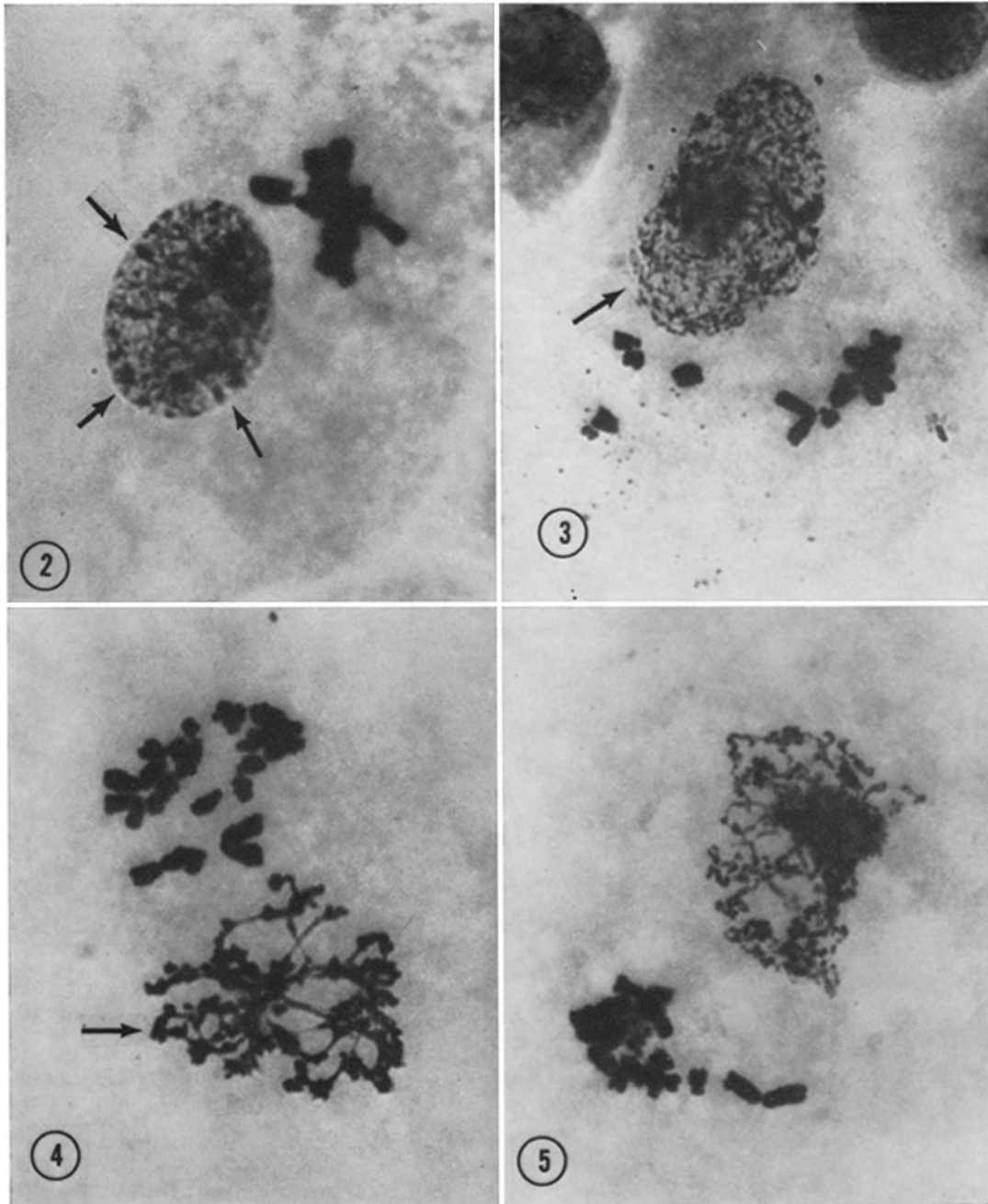


FIGURE 2 I-M cells immediately after cell division was completed. No conspicuous change has occurred in the interphase nucleus except for slightly enhanced heterochromatic regions (arrows). $\times 1950$.

FIGURE 3 I-M cell 5 min after cell fusion. Chromatin networks in the interphase nucleus are beginning to form (arrow). $\times 1950$.

FIGURE 4 I-M cell 10 min after cell fusion. The extremely condensed chromatin is in the form of a network in which the nucleolus is centered. The nuclear envelope is gone. Note one submetacentric chromosome-like structure associated with chromatin (arrow). $\times 1950$.

FIGURE 5 I-M cell 15 min after cell fusion. The bulk of the chromatin is in the form of long threads. The nucleolus still persists. $\times 1950$.

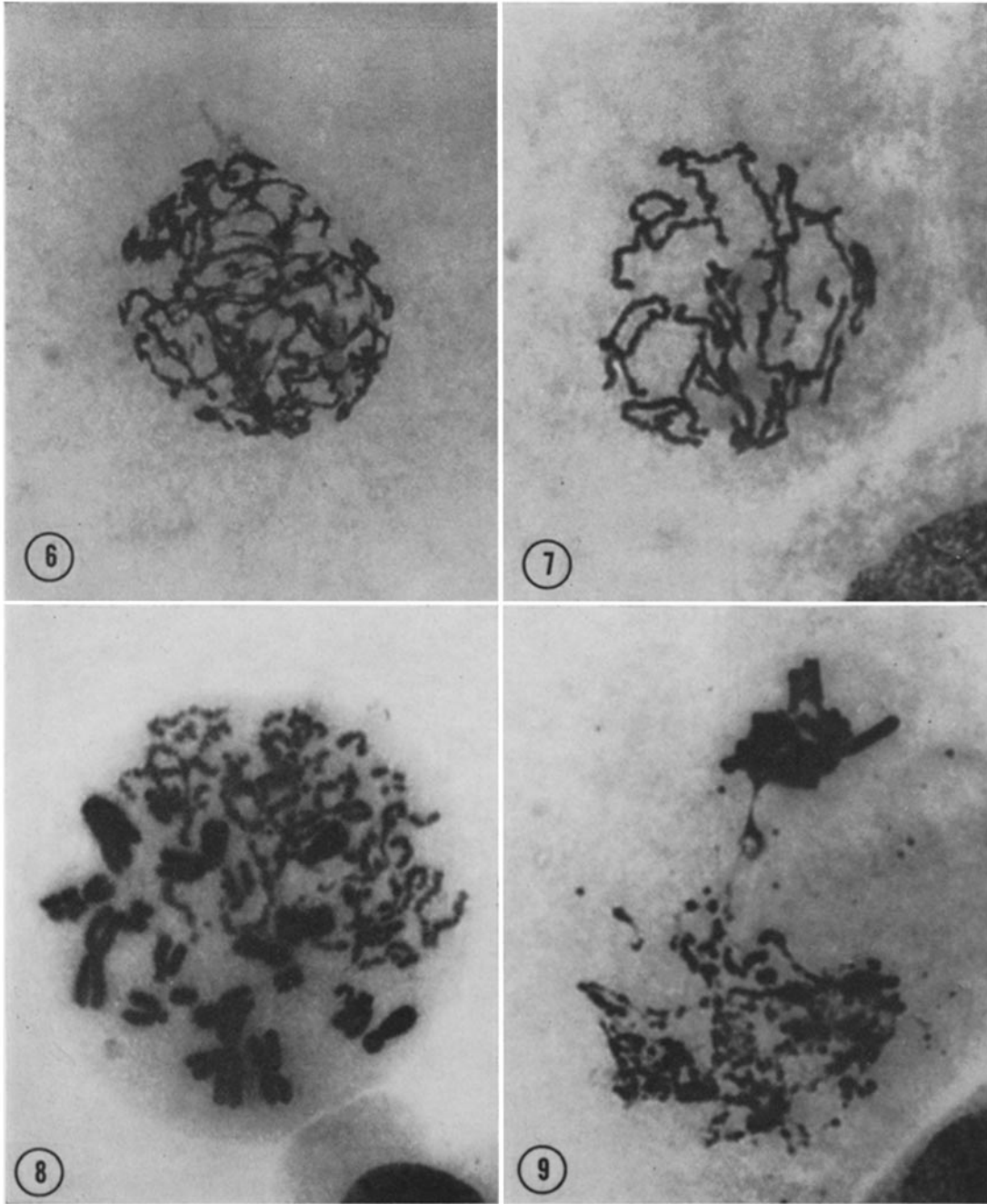


FIGURE 6 Nucleus in early normal prophase. $\times 2800$.

FIGURE 7 Nucleus in late normal prophase. $\times 2800$.

FIGURE 8 I-M cell 20 min after fusion. An appreciable amount of the interphase chromatin is in the form of sections smaller than the intact metaphase chromosomes. Fixed, after hypotonic treatment. $\times 2800$.

FIGURE 9 I-M cell 20 min after fusion. Directly fixed. $\times 2800$.

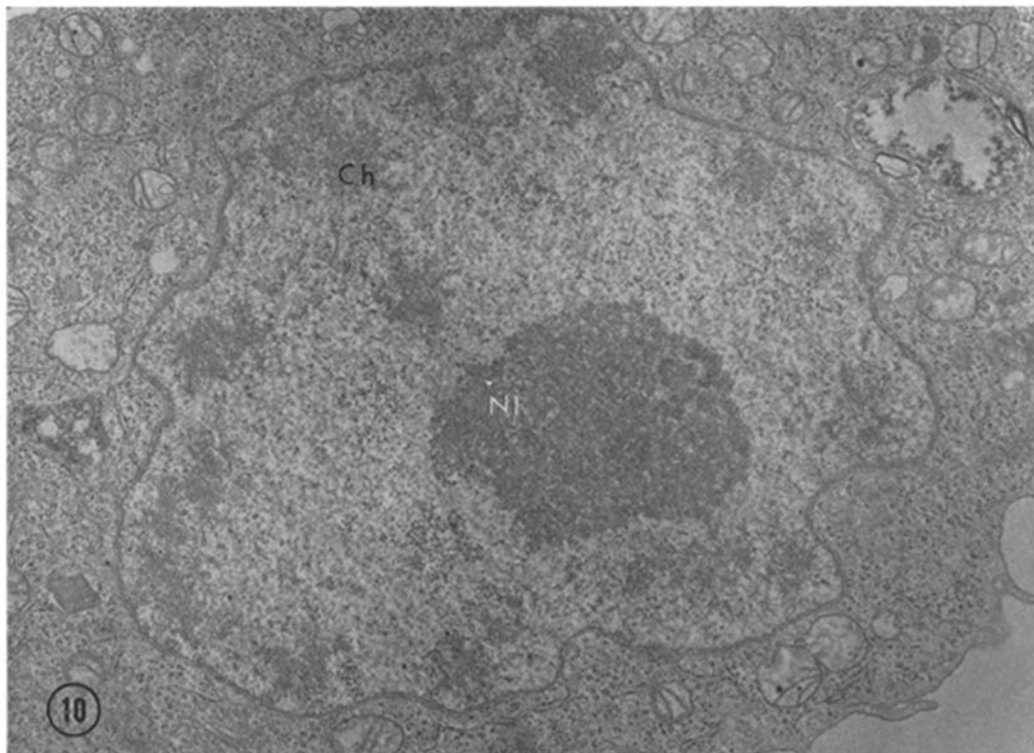


FIGURE 10 Electron micrograph of a cell in normal prophase. Condensed chromatin (*Ch*) is easily recognizable, some of it lying adjacent to the intact nuclear membrane. Elements of the nucleolus (*NI*) assume a granular form. $\times 10,800$.

rarely observed in the cytoplasm. These features are characteristic of prophase (5, 6, 24, 30)

Such features were observed exclusively in the I-M cells among the binucleate population 5–15 min after fusion, in accordance with the light microscope observations

The most pronounced change in the interphase nucleus of I-M cells was reached by 20 min after cell fusion. The interphase nucleus appeared to have been transformed into an assembly of irregularly oriented, condensed masses of chromatin. They were frequently associated with nucleolar elements and were clearly distinguishable from the metaphase chromosomes (not detectable in the light microscope) as shown in Fig. 12. Although not as compact as the chromatin of the metaphase chromosomes, the chromatin from the interphase nucleus did not have a fragmented appearance. Additional prominent features were the orientation of spindle tubules and the frequent appearance of a kinetochore (Figs. 12 and 13); the latter showed a unique structure

consisting of lampbrush-like filaments (Fig. 12) and was attached to a condensed mass of chromatin. Nuclear membrane fragments were scattered in the cytoplasm, and instead a quadruple-layered structure of membranous elements appeared (Figs. 12 and 13).

Thus, the ultrastructural changes that occurred in the interphase nucleus of I-M cells within 20 min after fusion were essentially similar to those occurring in normal prophase (5–7, 30)

Cells were synchronized, starting from metaphase, to each of the interphase stages, and fused with freshly prepared populations of metaphase cells. I-M cells were examined 10 min after fusion. The binucleate cells possessing G_1 and metaphase nuclei contained a chromatin network composed of numbers of single chromatids (Fig. 14), resembling early normal prophase (compare with Figs. 6 and 7). This is in agreement with the findings of Johnson and Rao with HeLa cells (14). The S-phase nuclei contained somewhat more complex networks of chromatin fibrils and aggre-

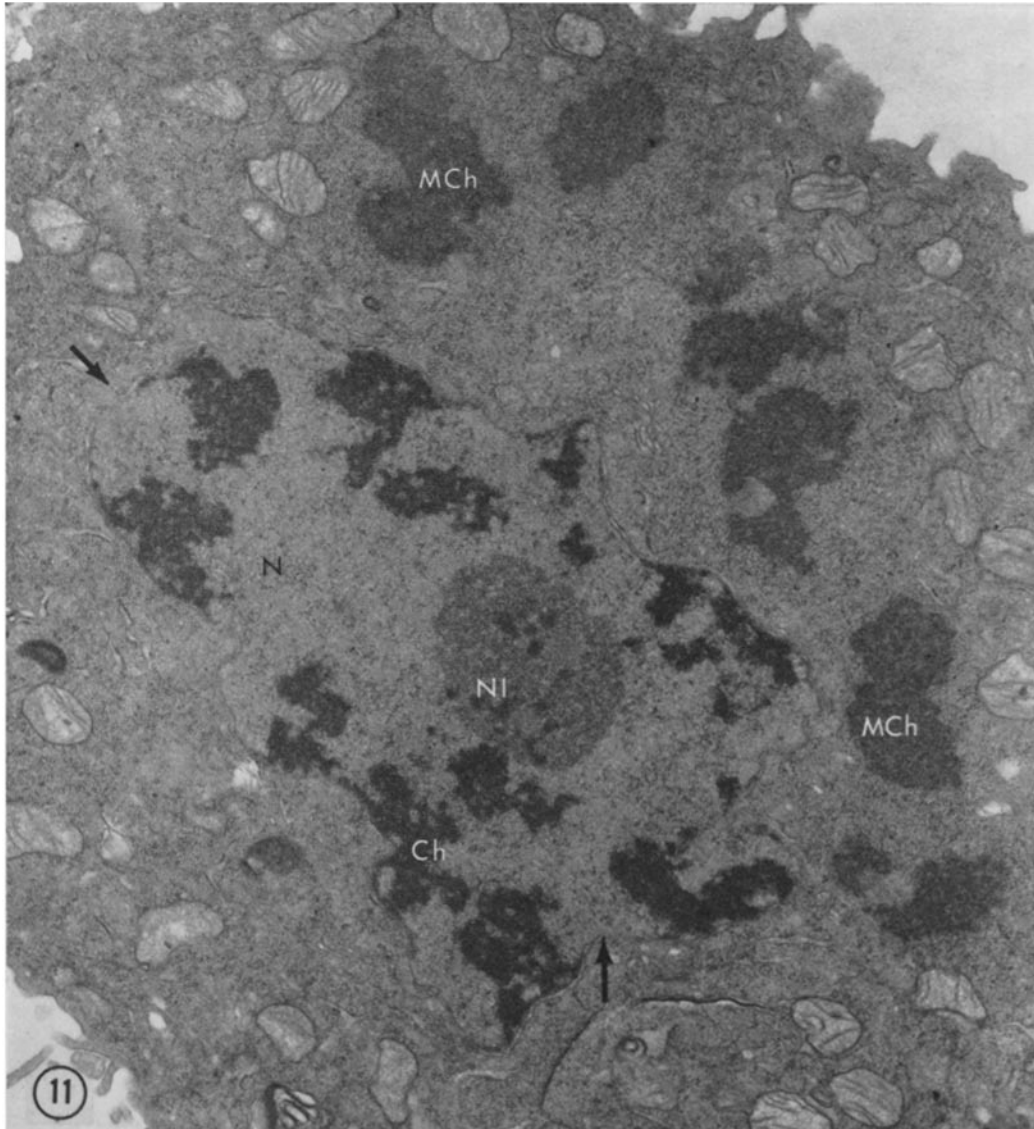


FIGURE 11 I-M cell 10 min after cell fusion. The chromatin (*Ch*) is distinctly condensed, some of it lying along the inner aspect of the nuclear membrane. Part of the nuclear membrane is disrupted (arrows). The nucleolus (*NI*) is composed predominantly of granular elements. The metaphase chromosomes appear intact (*MCh*). In the cytoplasmic area, polysomes are now rare. *N*, nucleus. $\times 14,400$.

gates and, perhaps, nucleoli (Figs. 15 and 16) Changes in the G_2 -nucleus were very similar to those of the normal nucleus in a G_2 -prophase transition (Figs. 4, 5, and 17). In a few instances a chromosome-like structure emerged among the condensed chromatin (Fig. 4). A conservative evaluation at this time cannot rule out the possibility that the structure may represent one of the pre-

formed metaphase chromosomes. Experiments with prelabeled metaphases or with heterokaryons of readily distinguishable karyotypic structures would have to be performed in order to decide whether chromatin condensation ultimately leads to the appearance of metaphase chromosomes.

Thus, although all interphase nuclei appear to be susceptible to induction of prophase, the fea-

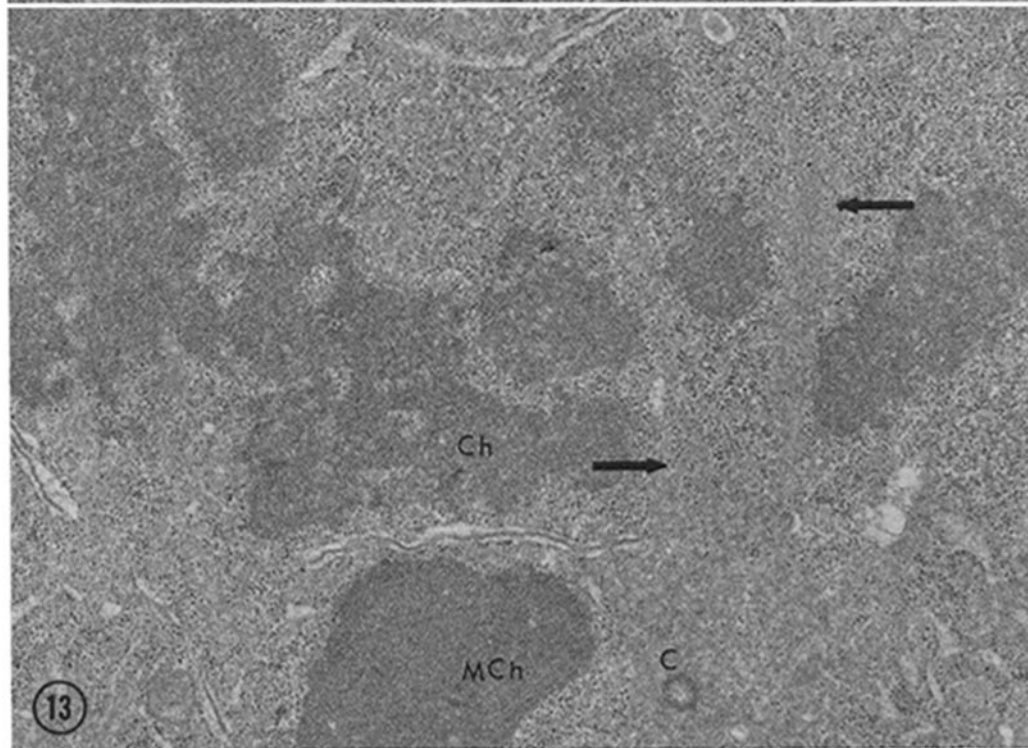
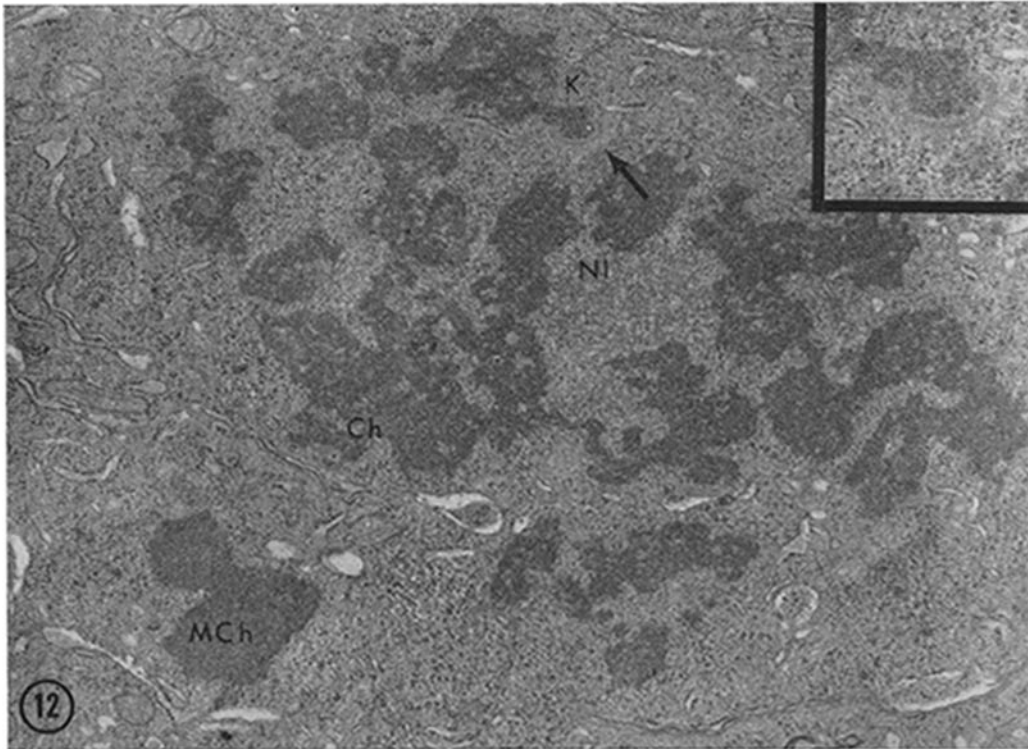


FIGURE 12 I-M cell at 20 min after cell fusion. Condensed chromatin (Ch) and a granular nucleolus (Ni) originating from an interphase cell. A kinetochore (K) is attached to the condensed chromatin (arrow). A metaphase chromosome (MCh) is present in this field. $\times 14,000$. The *insert* shows a further enlargement ($\times 28,000$) of the kinetochore area.

FIGURE 13 Another I-M cell 20 min after cell fusion. Several spindle tubules (arrows) stretch to the condensed chromatin (Ch). The degree of condensation is different between interphase chromatin and metaphase chromosomes (MCh). Note the centriole (C). $\times 12,000$.

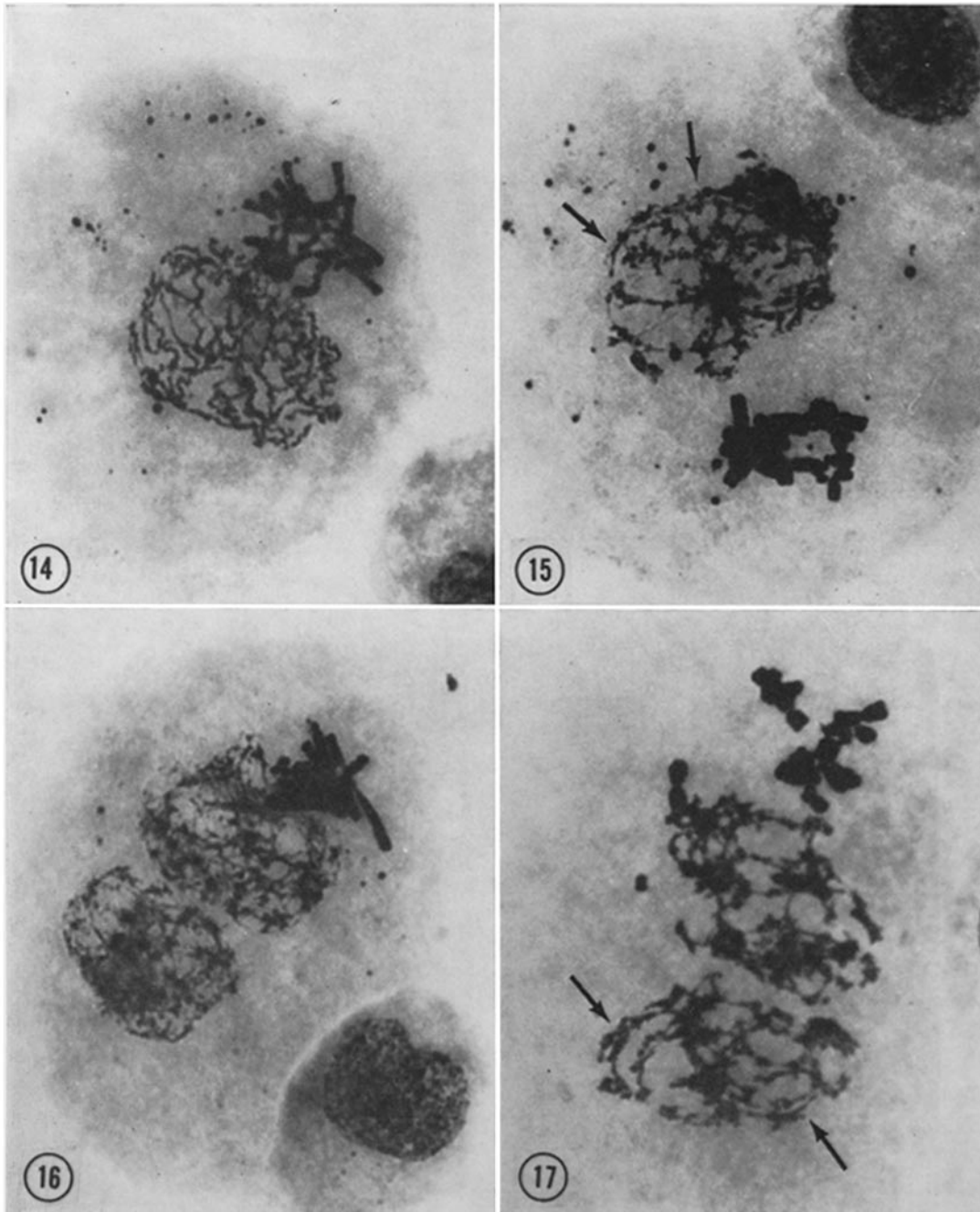


FIGURE 14 I-M cell containing a G_1 nucleus 10 min after cell fusion. The chromatin network is composed mainly of single chromatid-like structures. $\times 1700$.

FIGURE 15 I-M cell containing an S_1 nucleus 10 min after cell fusion: There is some suggestion of double chromatid-like structures (arrows). $\times 1700$.

FIGURE 16 Trinucleate cell containing one intact diploid set of metaphase chromosomes and two S-phase nuclei 10 min after fusion. Both S-phase nuclei appear to be in the same prophase-like stage. $\times 1700$.

FIGURE 17 Trinucleate cells containing one intact diploid set of metaphase chromosomes and two G_2 nuclei, 10 min after cell fusion. The condensed interphase chromatin forms a network, which is in part composed of double chromatid-like structures (arrows). $\times 1700$.

TABLE I
Release of Nuclear RNA

Cells	Time after cell fusion	Grain count (mean \pm sd)	
		Chromatin (%)	Cytoplasm
	<i>min</i>		
M* + intact I‡	0	41.5 \pm 8.2 (100)	18.0 \pm 4.5
M + prophasing I	10	29.2 \pm 4.8 (70.4)	29.0 \pm 6.3
M + intact I	20	44.2 \pm 6.8 (108.9)	17.7 \pm 6.3
M + P§	20	15.0 \pm 4.0 (36.1)	38.3 \pm 7.0
Two intact I	20	39.5 \pm 9.4 (95.2)	20.1 \pm 5.0
intact I	20	42.8 \pm 6.5 (103.1)	15.5 \pm 3.4
M¶		2.0	32.9 \pm 4.9

Grain counts were obtained on 100 cells in each experiment.

* Metaphase set of chromosomes.

‡ Interphase nucleus.

§ Pulverization.

|| Grain counts per one cell.

¶ Metaphase cells were obtained from log phase cells which were exposed to 10 μ Ci/ml of uridine-³H for 30 min and, subsequently, to 0.06 μ g/ml of Colcemid for 3 hr.

tures appear to depend on the interphase stage (G_1 or S or G_2) at the time of cell fusion

Release of Nuclear RNA and Protein during Prophasing

Prominent among the biochemical events occurring in normal mitosis is the extensive release of nuclear RNA and protein (25-27). The question as to whether the induced prophase also resembled normal prophase biochemically was examined. For this purpose, interphase cells obtained from log phase cultures were labeled in their nuclei for 30 min with 10 μ Ci/ml of precursors of RNA or protein, washed with fresh medium, and subsequently fused with a freshly prepared population of metaphase cells. Labeling time for RNA was not longer than 30 min in order to minimize cytoplasmic labeling, which could obscure any subsequent release of labeled material from the nuclei in I-M cells. The I-M cells were harvested periodically within 35 min after fusion. When fusion was just completed, at 10 min after viral treatment, no appreciable release of RNA from the interphase nuclei of I-M cells had occurred (Fig. 18 and Table I). On the contrary, a considerable amount of RNA was transferred to the cytoplasm from nuclei when they reached the prophase-like stage (Fig. 19 and Table I). By 20 min after fusion, the released RNA from chromatin amounted to as much as 64% of the presynthesized RNA (Fig. 20

and Table I). In a prophase-metaphase transition in normal mononucleate cells, almost all of the prelabeled RNA was found to transfer from the nuclei into the cytoplasm (Fig. 21 and Table I; compare to Fig. 18). A partial, though substantial, release of RNA from interphase chromatin in I-M cells might indicate that the process is not quite so efficient as that in normal mitosis.

In contrast to the clear-cut indication for the release of RNA, the results with proteins were not consistent. The results with lysine were statistically significant ($P < 0.01$), but those with arginine, tryptophane, and leucine were not (Table II). Accordingly, it is likely that a certain protein, preferentially consisting of lysine, such as histones, was selectively released from the chromatin.

DISCUSSION

We have observed that the alterations in the interphase nuclei of I-M cells simulate both morphologically and biochemically the changes that occur during normal prophase (3-7, 30). The morphological changes were exhibited by nuclei of cells in the G_1 , S, or G_2 phases before the appearance of CP. Events in this prophase induction, analogous to those in normal prophase, are: (a) appearance in the light microscope of chromatin networks; (b) a condensation of chromatin along the inner nuclear membrane, as observed in the EM; (c) a disruption of nuclear membrane; (d) possible attachment of spindle tubules to chromatin; (e)

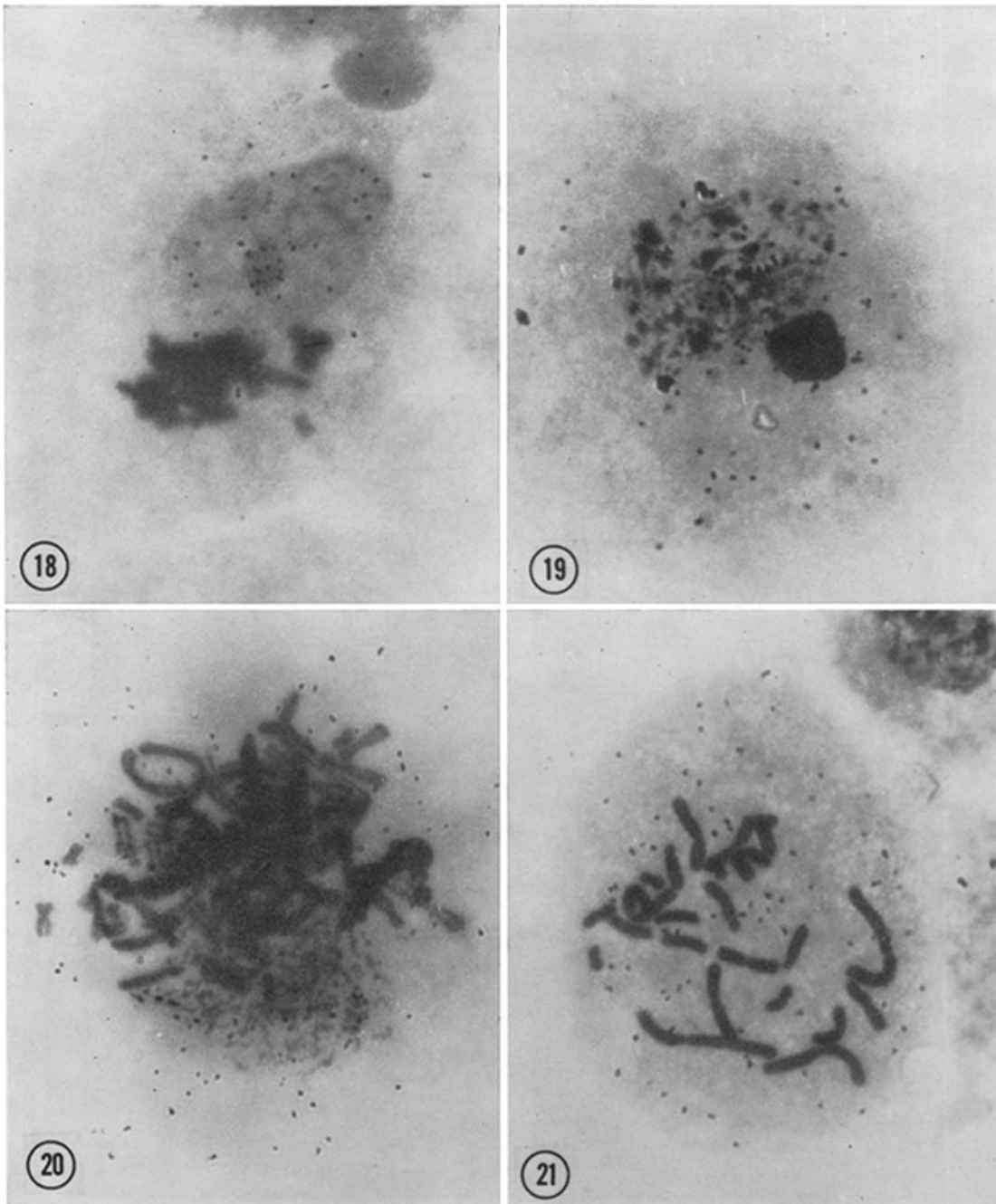


FIGURE 18 Radioautograph of an I-M cell that contains one intact set of metaphase chromosomes and an interphase nucleus that had been labeled with uridine- ^3H before cell fusion. Directly fixed immediately after cell fusion was completed. Almost all silver grains are located on the nucleus. $\times 1950$.

FIGURE 19 I-M cell 10 min after cell fusion, showing a transfer of silver grains from nucleus into cytoplasm. Directly fixed. $\times 1950$.

FIGURE 20 An I-M cell 20 min after cell fusion was treated with 15 mM sodium citrate and then fixed. Almost all of the silver grains have been transferred from the original interphase chromatin into the cytoplasmic area. $\times 1950$.

FIGURE 21 Release of RNA at the time of normal mitosis: Log phase cells were labeled with $10 \mu\text{Ci/ml}$ uridine- ^3H for 30 min, washed, and subsequently exposed to $0.06 \mu\text{g/ml}$ of Colcemid for 3 hr. The silver grains are predominantly in the cytoplasm. $\times 1950$.

TABLE II
Release of Nuclear Protein

³ H-precursor	Grain count (mean ± sd)			
	Chromatin		Cytoplasm	
	Intact (I)	Pulverized (P)	With I	With P
Tryptophan	8.9 ± 2.0	8.1 ± 2.7	15.3 ± 3.1	14.3 ± 3.8
Arginine	17.9 ± 3.5	12.5 ± 2.8	30.2 ± 4.2	27.3 ± 3.0
Leucine	13.6 ± 3.0	11.3 ± 2.5	28.2 ± 3.9	28.5 ± 6.0
Lysine	14.7 ± 4.1	8.2 ± 2.8	15.6 ± 3.0	24.1 ± 4.8

50 I-M cells were examined for each amino acid

the appearance of a structure resembling the kinetochore, (f) dissociation of polysomes; and (g) a release of nuclear RNA (and probably protein) into the cytoplasm

Earlier results with DON cells in this laboratory (17) established that protein synthesis in late G₂ before fusion was necessary if the resulting metaphase cell was to induce prophase in an interphase nucleus after it had fused with an interphase cell. When that finding and the present results are considered together, the conclusion is inescapable that the metaphase cell contains a factor(s) that can initiate normal mitotic events in G₁, S, or G₂ nuclei. A similar conclusion, based on observations made with the light microscope only, was applied to fused HeLa cells (14)

The results pose the interesting question, among others, as to how these factors become inactivated so as to permit the metaphase cell to proceed into G₁. It was observed in this laboratory (11) that aging the cells in the presence of Colcemid can cause inactivation of the ability to induce prophase, while allowing the interphase cell nucleus and/or cytoplasm to promote the formation of a nuclear envelope

Additional inductive effects by the nucleus and/or cytoplasm of one cellular phase on the nucleus of another have been studied by other workers, notable examples being the initiation of DNA synthesis in a G₀ or G₁ nucleus after fusion with an S phase cell (9, 28) or by extracts of S-phase cells (8). Proteins that are probably specific for the G₁ phase have been described (31). All of these studies emphasize the reality of distinct biochemical factors that are responsible for specific mitotic events

The morphological and biochemical observations lead us to believe that cell fusion, at least as applied to DON cells in our laboratory, can be used as a tool in attempting the biochemical char-

acterization of factors responsible for a variety of mitotic events

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