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MACRONUCLEAR CYTOLOGY OF SYNCHRONIZED TETRAHYMENA PYRIFORMIS*

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Elliott, Kennedy and Bak ('62) and Elliott ('63) followed fine structural changes in macronuclei of Tetrahymena pyriformis which were synchronized by the heat shock method of Scherbaum and Zeuthen ('54). Using Elliott's morphological descriptions as a basis, we designed our investigations with two main objectives: First, to again study the morphological changes which occur in the macronucleus of Tetrahymena synchronized by the heat shock method. The second objective was to compare these observations with Tetrahymena synchronized by an alternate method recently reported by Padilla and Cameron ('64). Therefore, we were able to compare the results from two different synchronization methods and to contrast these findings with the macronuclear cytology of Tetrahymena taken from a logarithmically growing culture. Comparison of cells treated in these three different ways enables us to evaluate the two different synchronization methods and to gain more information on the structural changes taking place in the macronucleus of Tetrahymena as a function of the cell cycle.

Our observations were confined primarily to nucleolar morphology. The results indicate that cells synchronized by the Padilla and Cameron method more closely resemble logarithmically growing Tetrahymena in the macronuclear structure than do cells obtained by the Scherbaum and Zeuthen synchronization method.

MATERIALS AND METHODS

Tetrahymena pyriformis (strain HSM) was grown axenically on a medium of 1.5% proteose peptone (Difco) plus 0.1% liver extract (Nutritional Biochemical Corp.). Cells were transferred daily to fresh medium to assure logarithmic growth. Unless otherwise noted, cells were maintained at 29° C.

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Five samples for electron microscopy were taken in the Padilla-Cameron synchrony procedure at about 45-minute intervals at the beginning, middle and end of the warm period. Other samples were taken of stationary and log phase cells.

OBSERVATIONS

Types of macronuclear bodies

Our phase contrast and electron microscopic observations permitted resolution of three different types of macronuclear bodies. 1) Small chromatin bodies approximately 0.1 μ in diameter (Figs. 1a, b, c, 5, 9). The chromatin bodies are evenly distributed throughout the entire nucleus. In electron micrographs, these chromatin bodies show up as numerous electron opaque bodies (Fig. 1a, b, c; also see Roth and Minich, '61; Elliott, Kenney, and Bak, '62; Elliott, '63; Pitelka, '63; Swift, Adams, and Larson, '64; Cameron and Guile, '64, '65). These chromatin bodies are interconnected by a coarse network of fibrillar material. 2) A second type of nuclear bodies that are larger in size (0.3-1.0 μ in diameter) and generally are closely associated with and equally distributed over the inner surface of the nuclear envelope. These bodies, which are often cup-like in form, have been described as nucleoli by several authors (Roth and Minich, '61; Elliott, Kennedy, and Bak, '62; Elliott, '63; Pitelka, '63; Swift, Adams, and Larson, '64; Cameron and Guile, '64, '65). The open portion of the nucleolus is generally oriented towards the center of the nucleus (Fig. 1a). Nuclolar fine structure consists of a granular cortical layer and a less dense core portion (Fig. 1b; also see Elliott, '63; Swift, Adams and Larson, '64). Often one or more small dense bodies are observed in the concavity of the nucleolus (Fig. 1a). Electron microscopic autoradiography combined with pulse exposure to the specific DNA precursor, tritiated thymidine,

make us believe that these small dense bodies in the concavity of the cup-shaped nucleoli are nucleolar organizers (unpublished work by Miller and Stone). This belief is based on the fact that at one point in the cell cycle the dark dense bodies in the concavity of all nucleoli appear to replicate DNA synchronously and out of phase with the DNA replication in the rest of the nuclear chromatin. 3) A third and larger sized nuclear body (1-4 μ in diameter) is found in the macronucleus during certain growth conditions. Cameron and Guile ('64, '65) found that large nuclear bodies of this type arise from fusion of nucleoli as Tetrahymena entire stationary growth phase. Apparently the granular component of the nucleolus is eventually lost during the fusion process so that what remains in the large fusion body is the fibrous core portions of the original nucleoli (see Fig. 1a, b, c). Although we cannot be absolutely certain, we believe that these fusion bodies are the same as the "RNA bodies" previously described by Elliott, Kenney and Bak ('62) and Elliott ('63). We have never observed these large bodies in the macronucleus of logarithmically growing Tetrahymena.

The macronucleus of logarithmically growing cells

Both the chromatin bodies and nucleoli were always present in log cells, but the large fusion bodies were never observed in these cells. The macronuclei of Tetrahymena synchronized by the Padilla and Cameron procedure did not differ structurally in any noticeable way from the macronuclei of logarithmically grown cells.

Changes in the macronucleus during the Scherbaum and Zeuthen heat-shock synchronization procedure

During heat shocks the nucleoli begin to fuse (Figs. 6, 7, 8, and 9), but the fusion is not entirely complete, that is to say, that single nucleoli

can still be observed in heat synchronized cells. Fusion of nucleoli has been described as the normal process which occurs at the time a culture of Tetrahymena enters the early stationary phase (Figs. 1c, 4; also see Cameron and Guile, '64, '65). Since four-day-old cultures were used in Elliott's electron microscopic study of heat-shocked cells, it is likely that the "RNA bodies" that Elliott et al. ('62) described corresponded to the nucleolar fusion bodies that typically occur at stationary phase.

We were then led to ask if the synchrony method used by Elliott could by itself induce fusion of nucleoli into larger bodies. To test this possibility we started the heat-shock synchrony procedure with log phase cells which were known not to contain the large nuclear fusion bodies. The observations show that nucleolar fusion could be induced in log phase cells after heat shocks (Figs. 7, 8, 11) and that heat shocks of four-day-old cultures (early stationary phase cells) lead to the increased fusion of nucleoli. We failed to detect a rhythmic pattern of disintegration of fusion bodies during the heat shocks as described by Elliott, Kennedy and Bak ('62) or Elliott ('63). We were able to discern a pattern of macronuclear events at the end of heat shocks much like that described by Elliott et al. ('62). At the end of heat shocks, the fusion bodies within the macronucleus move to the periphery of the nucleus, then flatten somewhat against the nuclear envelope (Figs. 10 and 11). The fusion bodies progressively disaggregate into individual nucleoi and begin to evenly distribute against the nuclear envelope (Fig. 12). Not all of the nuclear fusion bodies subdivide during the interval before the first division and some are carried through the first division into the daughter nuclei (Fig. 13). During the course of the next few cell divisions all of the fusion bodies disappear. Careful observations of cells throughout the heat shock

synchronization procedure sometimes showed an outward bulging at one or a few points along the nuclear membrane (Figs, 10, 11), but no separation of macronuclear blebs as suggested by Elliott et al. ('62) and Elliott ('63) occurred at any time, even though individual cells were observed for periods of up to one hour. Fig. 14 summarizes the sequence of macronuclear events during heat shock synchronization of logarithmically growing cells.

CONCLUSION AND SUMMARY

Heat shock and stationary phase conditions both cause fusion of nucleoli. In both cases the process is reversed when the cell is returned to physiological growth conditions. There is no fusion of nucleoli in the cell cycle of logarithmically growing cells. The temperature shifts employed in the Padilla and Cameron synchronization method (12.5° C to 27.5° C and back to 12.5° C) did not cause nucleoli to fuse. Therefore it may be concluded that this synchronization method maintains cells in a condition more closely resembling log phase cells than does the Scherbaum and Zeuthen synchronization method.

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LEGENDS

Explanation of symbols used in the figures of this report: CB - chromatin bodies, N - nucleolus, FB - fusion bodies, MI - mitochondrion, MIC - micronucleus, NM - nuclear membrane, CF - cleavage furrow, OA - oral area, CV - contractile vacuole, NO - nucleolar organizer, GC - granular cortex of nucleolus, and P - pores in the nuclear membrane.

Figures 1a, b, and c. Electron micrographs of Tetrahymena pyriformis strain HSM. Fig. 1a shows the nucleolar organizers NO; in Fig. 1b the arrows point to the granular cortex of the nucleolus, GC; Fig. 1c shows the nuclear fusion body (FB) found in the macronucleus of a cell which was taken from a four-day-old culture. Notice the pores (P) in the nuclear envelope. X14,000, X18,000, and X16,000, respectively.

Figures 2-13. All phase contrast micrographs of T. pyriformis magnification X3,500.

Figure 2. Phase contrast micrograph of a squash preparation of Tetrahymena macronuclei. Numerous nucleoli are seen associated with the nuclear envelope.

Figure 3. Same as Figure 2 but the cell has not been squashed.

Figure 4. This is a cell taken from the early stationary phase (3 days postinoculation); notice that the nucleoli are aggregating (arrows) and also the granular nature of the nucleoplasm which is due to the numerous chromatin bodies. Rod shaped mitochondria are seen in the cytoplasm.

Figure 5. A log phase cell showing the micronucleus (arrow) lying in a macronuclear pocket. Focus is half-way through the macronucleus; therefore one sees mostly the chromatin bodies.

Figures 6-13. Series of phase contrast micrographs of macronuclear changes which occur before, during and after the Scherbaum-Zeuthen method of Tetrahymena synchronization.

Figure 6. A log phase cell prior to heat shocks.

Figure 7. Cell during first heat shock.

Figure 8. Cell during second heat shock.

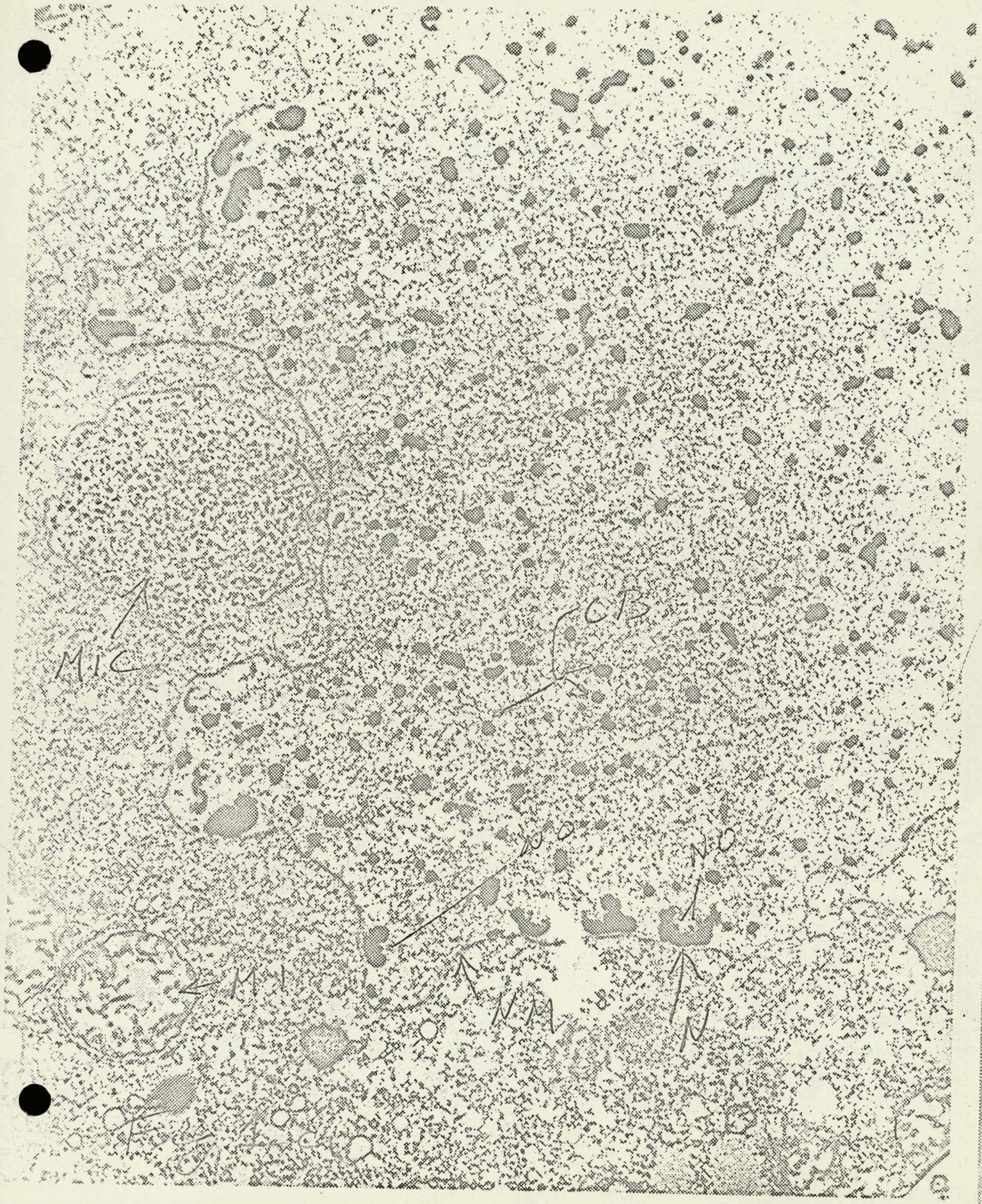
Figure 9. Cell after fourth heat shock; notice the two large fusion bodies, one has the appearance of containing vacuoles.

Figures 10 and 11. After one hour after last heat shock; notice the fusion bodies (arrows) flattening against the nuclear membrane.

Figure 12. Ninety minutes after the last heat shock; notice that nucleoli are reappearing although they are in clumps against the nuclear membrane. Some fusion bodies have not yet disaggregated. The micronucleus (arrow) and oral area (O) can be seen in this cell.

Figure 13. In this cell the macronucleus has already divided and the cleavage furrow is visible. The fusion body (arrow) has not disaggregated prior to the first cell division and is therefore carried to one of the resulting daughter macronuclei. Other fusion bodies are flattened against the nuclear envelope.

Figure 14. A diagrammatic presentation of macronuclear events during heat shock synchronization of logarithmically growing cells.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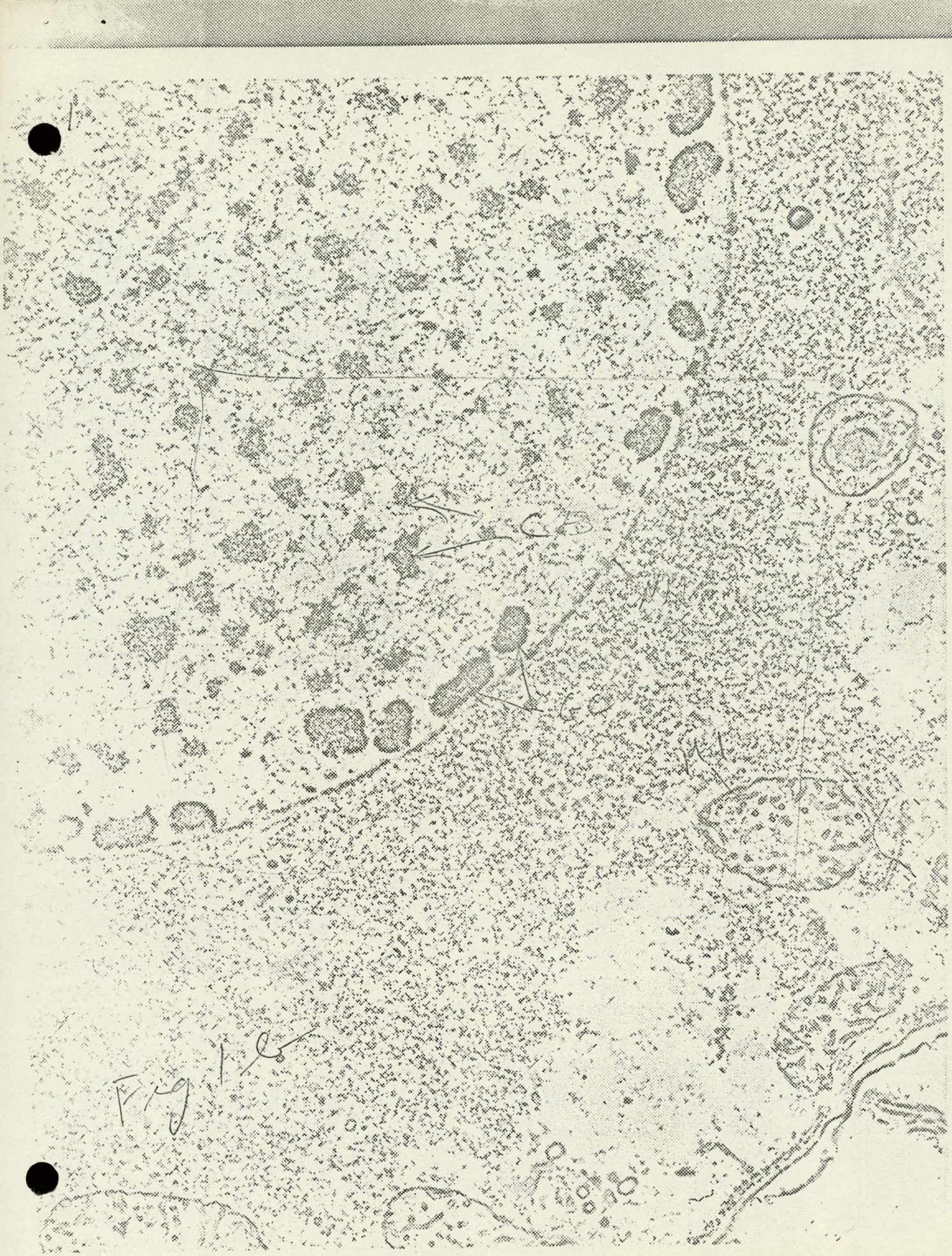
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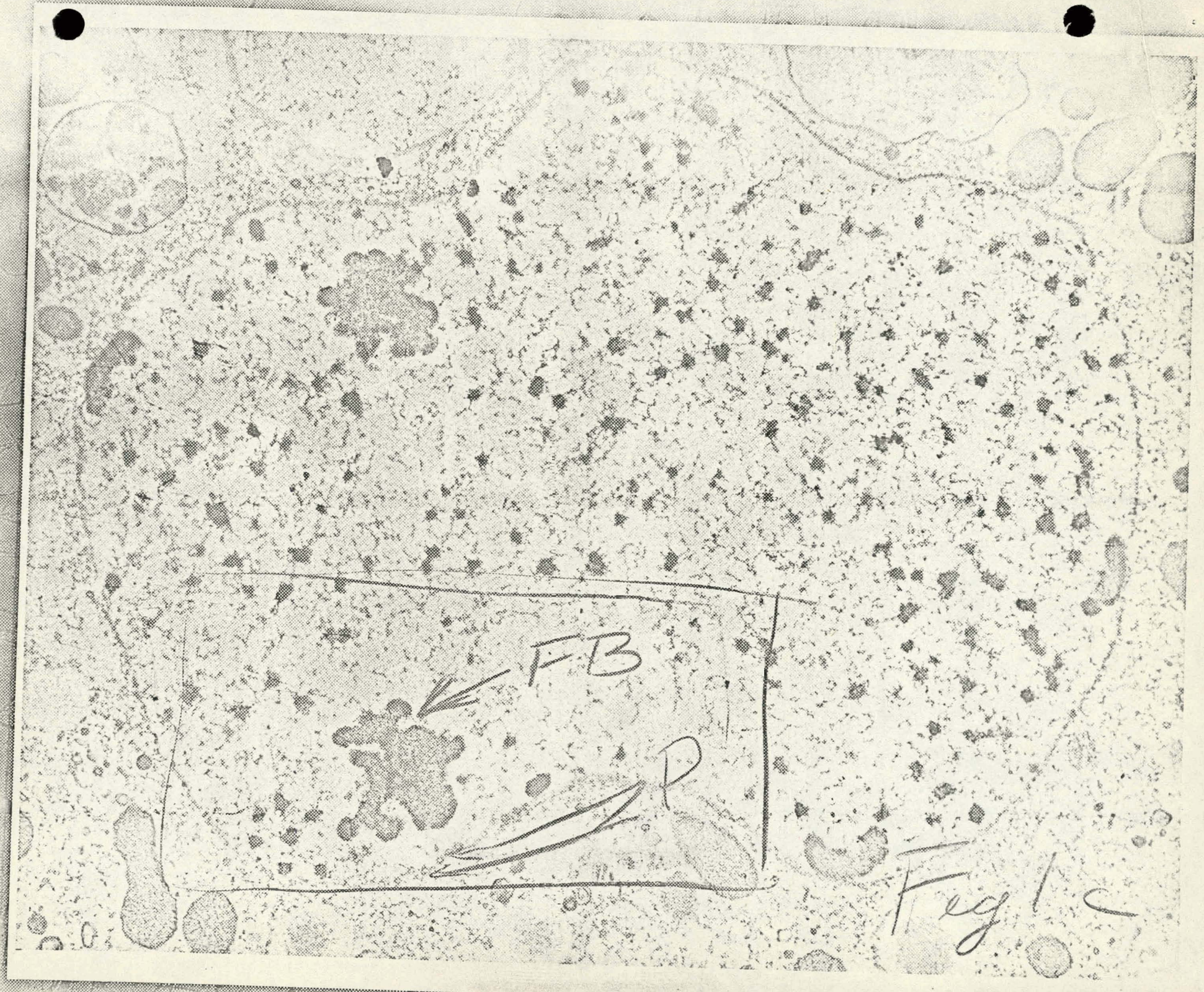
Fig 1



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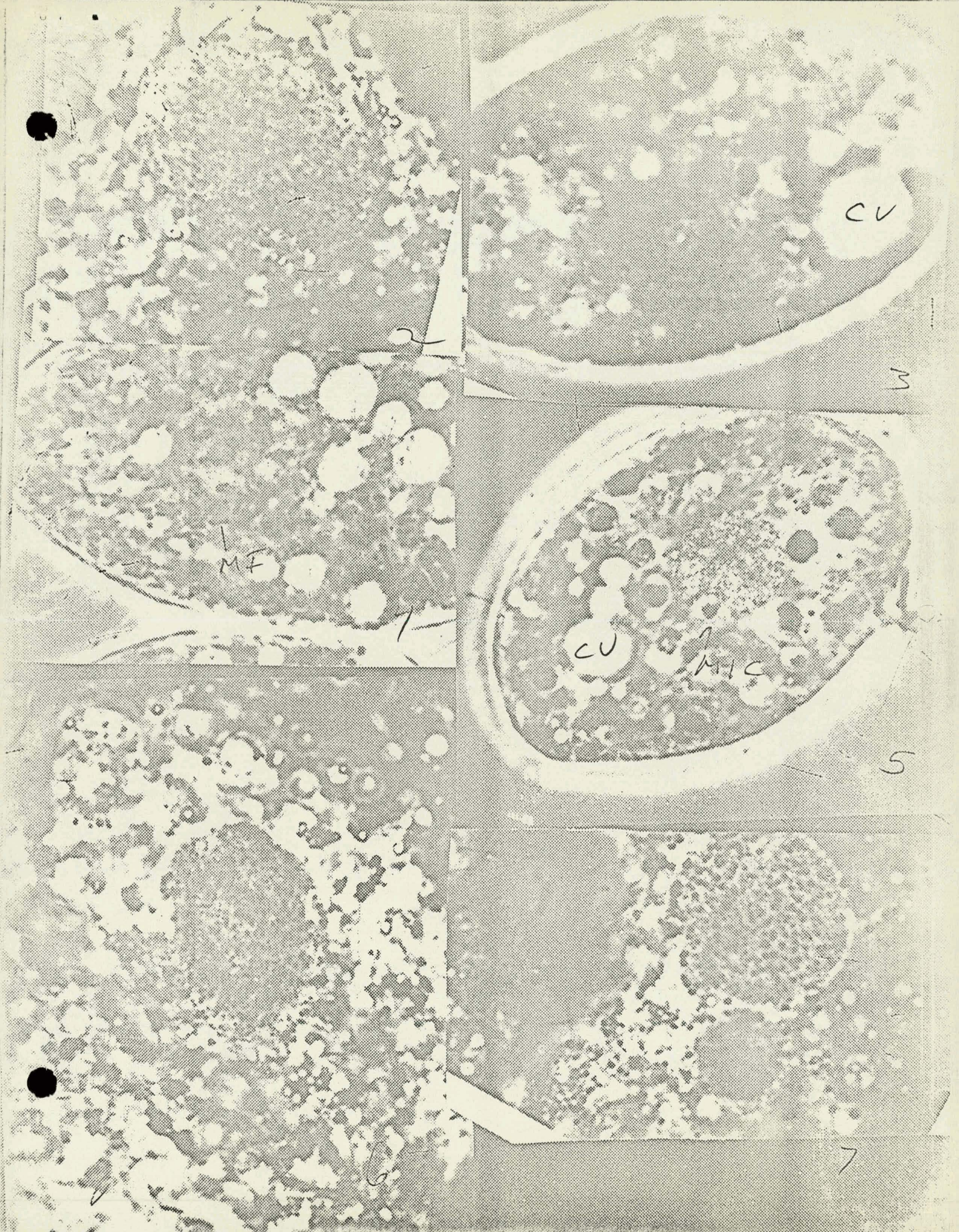
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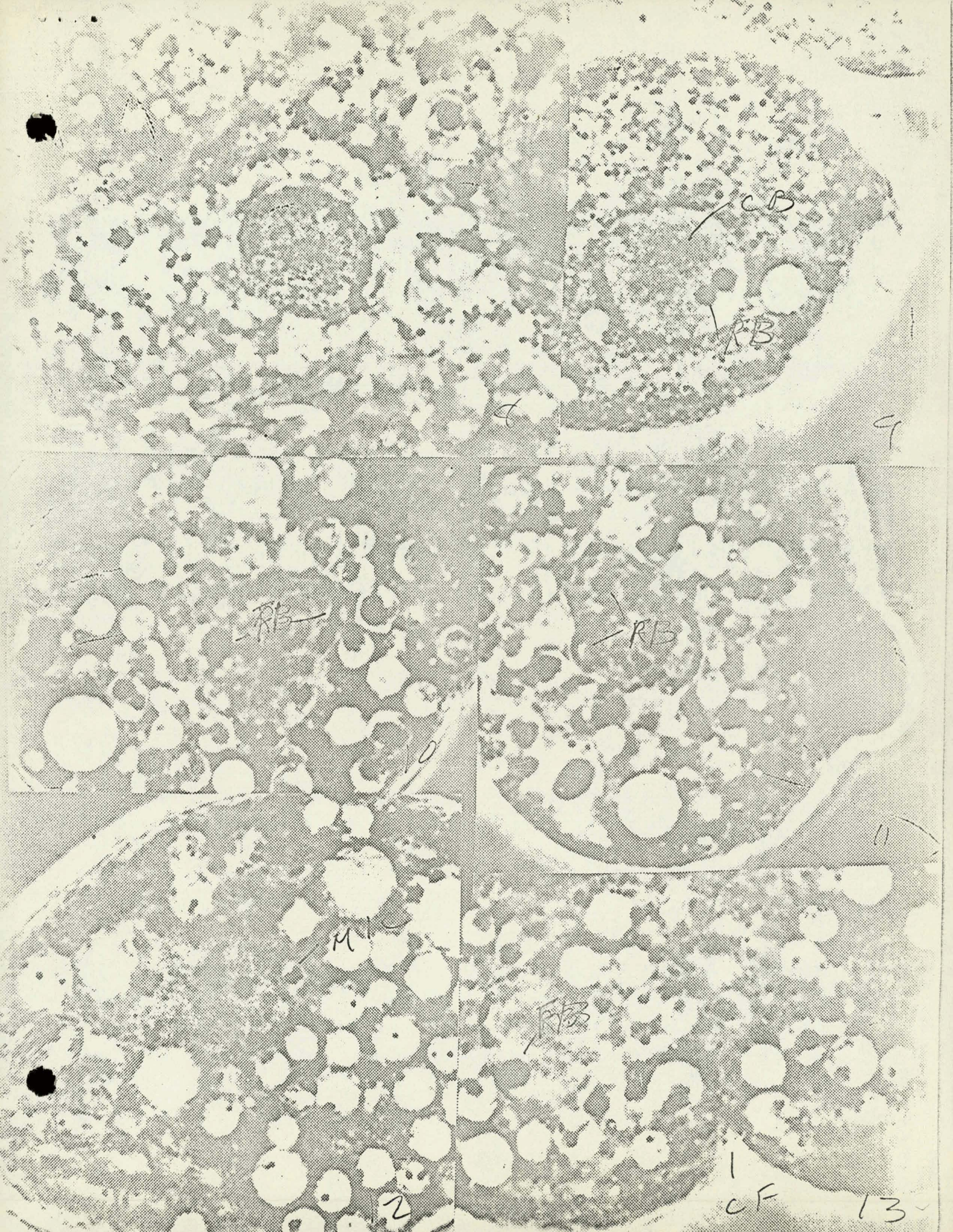
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Macronuclear events during heat shock synchronization of logarithmically growing cells

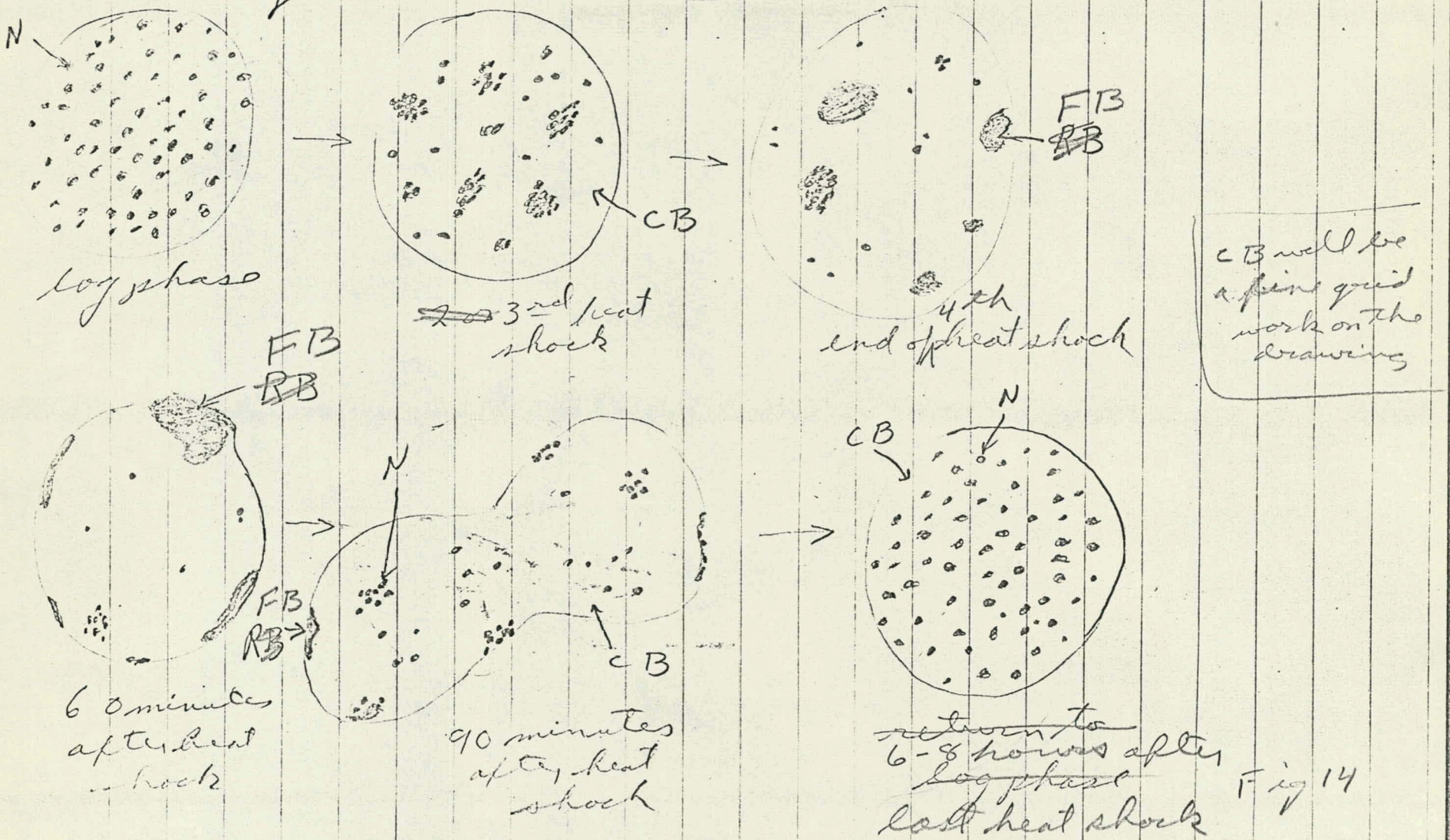

Fig 14

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N nucleoli

RB ~~Fuzzy Bodies~~

CB chromatin bodies