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The Mutagenic Action of Caffeine in Higher Organisms[1]

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Introduction

Caffeine is a purine, 1, 3, 7-trimethyl xanthine. Since it is consumed in great quantities by man, the question concerning whether it is a mutagen for man is of great significance. There is now good evidence that caffeine is mutagenic not only in bacteria (4, 5, 7, 9, 18), fungi (6, 41), and plants (11, 12), but equally mutagenic in *Drosophila melanogaster* [2, 16, 25, 29; negative reports, (40)], in mice [Kuhlmann and Ostertag 1968, unpublished; negative report, (15); ambiguous results, (3)], as well as in human cells *in vitro* (24, 26, 28; Jacobson, unpublished). There is some evidence that caffeine inhibits dark repair in bacteria (38) as well as in mammalian cells (33; Jacobson, C., unpublished). Caffeine is teratogenic in mice if high doses are applied (17). There is no evidence that caffeine is involved in carcinogenesis. We have good evidence from studies with *Drosophila melanogaster* that caffeine also causes somatic damage by breakage of chromosomes in somatic cells (29). By analogy with the action of radiation, which causes the same type of somatic damage (23; Ostertag, unpublished data), we have good reason to assume that caffeine induces premature aging in Drosophila.

Effect of Caffeine on Cell Growth and Nucleic Acid Metabolism in Mammalian Cells

Caffeine inhibits the growth of HeLa cells in concentrations above 200 μg/ml (26). At the same concentration we find inhibition of RNA synthesis, but not of DNA synthesis (Chart 1). At very low doses there is possibly a stimulation of DNA synthesis. By following the kinetics of the inhibition of DNA and RNA synthesis at a dose of 500 μg/ml, DNA synthesis proceeds normally up to 20 hours, while inhibition of RNA synthesis starts at about 2–3 hours after caffeine application and is then about 50% (Chart 2) (Heege, E. M., unpublished results). At these dose levels of caffeine we would therefore expect a change in the ratio of DNA/RNA. We do not know whether the synthesis of any of the three major RNA species–transfer RNA, messenger RNA (mRNA), ribosmal RNA–is preferentially inhibited. Recently, inhibition of DNA polymerase activity by caffeine has been observed in human cells in an *in vitro* assay at concentrations above 500 μg/ml after 48 hours. If mitomycin C or aflatoxin B are added to these cultures, DNA polymerase activity is elevated at certain concentrations by about 50%. This elevation of DNA polymerase activity is inhibited by caffeine concentrations above 200 μg/ml (39). Recently we checked the period of DNA synthesis after exposure of HeLa cells to caffeine. In HeLa cells one division cycle normally lasts 20.5 ± 1 hr. DNA synthesis (S phase) takes 6 hours; a lag phase then follows without DNA synthesis (G-2) for approximately 4.6 hr. before mitosis, which takes 1.1 hr. The rest of the division cycle is the G-1 phase (32). We added 1% caffeine for two hours at 0 time, removed the caffeine, and then exposed the cells to thymidine-^{3}H for 10 minutes at various times after caffeine exposure in order to label the newly synthesized DNA. Twenty-four hours after 0 time, the cells were fixed and autoradiographs were prepared. For each metaphase we determined whether there was any label incorporated into the chromosomes. Exposure to these high concentrations of caffeine for two hours affects the cell cycle only slightly. The time between DNA synthesis and the next metaphase is about one hour longer than in the untreated cells (Heege, E. M., unpublished results) (Chart 3).

Morphology of Cells Treated with Caffeine

HeLa cells lose their attachment to the glass of the Petri dishes if treated with more than 100 μg/ml of caffeine. They also get more spherical as if they had been treated with trypsin (Figs. 1, 2). If 1% caffeine is used, there are extensive changes of the fine structure of HeLa cells (27). The main damage is to the nucleus, which starts to segment; nuclear fragments move across the cytoplasm and are found outside the cell membrane. This expulsion of nuclear material is effected only by caffeine and has not been found after exposure to other damaging influences. It is perhaps similar to the enucleation process during normal erythrocytic maturation (Figs. 3–5). The extrusion of nuclear fragments might be a consequence of the unbalanced nucleic acid metabolism and the relative lack of mitotic inhibition which is found after caffeine exposure. Agents such as actinomycin D, mitomycin C, and chromomycin A3, which also induce imbalance of nucleic acid synthesis (in one way or another), are very potent inhibitors of mitosis in mammalian cell cultures (25), yet they do not induce expulsion of nuclear fragments.

By high resolution autoradiography we could show that those cells which respond to caffeine application by fragmentation of their nuclei have lost their ability to incorporate thymidine-^{3}H. In the same culture there are other cells which incorporate thymidine-^{3}H to the same extent as in control

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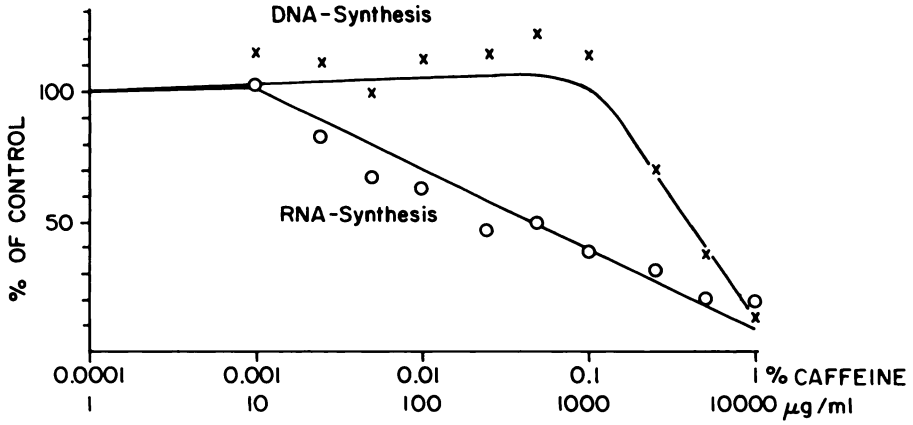

Chart 1. Inhibition of DNA/RNA synthesis by caffeine.

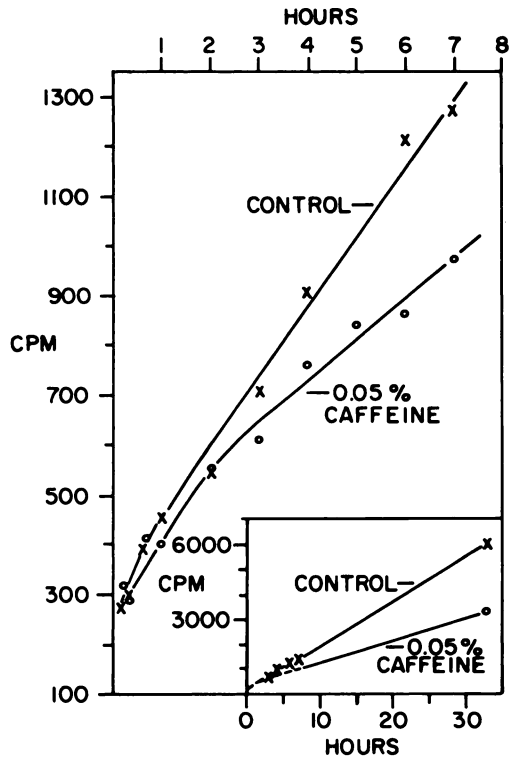

Chart 2*a*. Inhibition of RNA synthesis by 0.05% caffeine (^{14}C orotic was added at time 0).

experiments (Fig. 6). After caffeine treatment almost all of the DNA is perinuclear. This has also been found after X-irradiation of mammalian cells (Figs. 7, 8).

Chromosome Breakage by Caffeine

Type of Breaks Induced by Caffeine. As can be seen in Figs. 9 and 10 and Table 1, caffeine, when applied to human cells in culture, does not induce many translocations, rings, and other rearrangements that require fusion of the broken ends. The rearrangements, if present, are often in clusters in one single cell. We therefore have good reason to assume that these rare rearrangements are induced by a secondary, physiologic mechanism. We conclude that caffeine, in contrast to all other agents that cause chromosome or chromatid breakage, does not induce such sticky breaks. The rejoining mechanism, which is possibly part of the repair mechanism, seems to be blocked for caffeine-induced chromatid breaks. In addition to normal breaks, we quite often find lesions of a long stretch of chromatin. These cannot be caused by two breakage events, as nonterminal deletions normally are, but rather must be caused by a failure of synthesis, possibly combined with enzymatic removal of the DNA strand that failed to replicate.

Dose Response. Caffeine does induce breaks in human chromosomes even in doses below 500 µg/ml (Chart 4, Table 1). Various doses were applied for one hour. The caffeine-containing medium was then removed, the cells were washed with caffeine-free medium, and caffeine-free medium was added for another 23 hours. The cells were then fixed and the metaphases examined. There is an increase of the caffeine-induced breakage correlated to the dose. This increase is linear in the lower dosage ranges and more than linear at very high doses. To see whether the greater than linear increase in breakage at higher dose level was due to the same mechanism that induced breakage at a lower dose of caffeine, we analyzed cells with similar chromosome number for the number of breaks per cell at the higher dose level.

We would expect that there is a very high frequency of breakage in the same cell if at a certain threshold a physiologic chain of reactions is initiated that results in secondary chromosome breakage, perhaps due to release of DNases. We therefore would expect two types of cells, those showing a very high frequency of breakage and those having much less breakage. The latter type would be linearly dose dependent, and the former would be absent at a low dosage. Corresponding to expectation, we found that 1% caffeine exposure caused a deviation in the distribution of breaks per cell from the average number of breaks expected according to the Poisson distribution (Chart 5). There are many more cells with a high number of breakage per cell than expected (25 breaks and more per cell; expected 0.45%, found 13.75%). The dosage

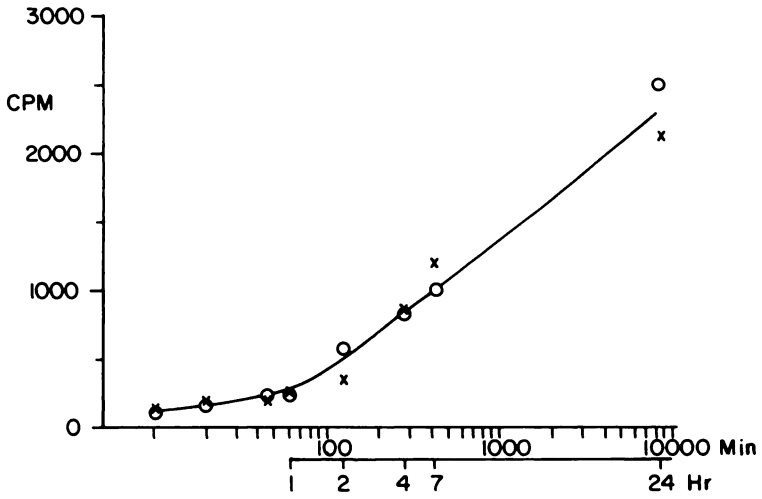

Chart 2*b*. Lack of inhibition of DNA synthesis by 0.05% caffeine, (Thymidine-^{14}C was added at time 0). -o-o-o-, control; -x-x-x-, 0.05% caffeine.

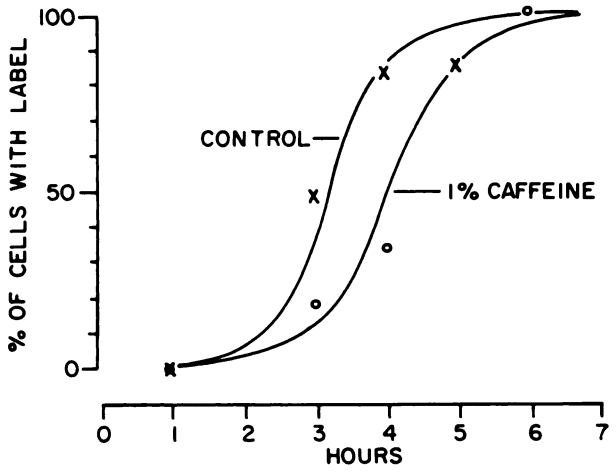

Chart 3. Time between DNA synthesis and metaphase.

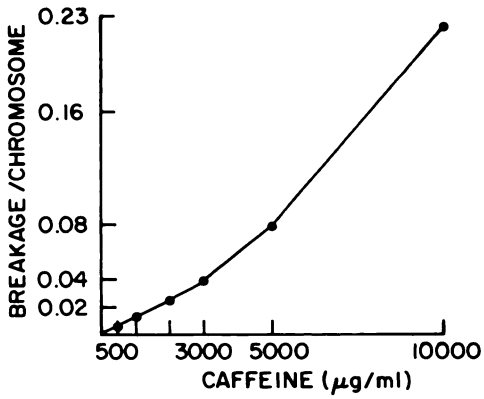

Chart 4. Breakage induced by caffeine in HeLa cells. Dose dependence [Ostertag *et al.* (26)].

Table 1

Caffeine (μg/ml)	Number of chromosomes	Chromatid + isolocus breaks		Translocations	Dicentrics	Rings
		Number	per chromosome			
0	4187	4	0.0009			
500	2754	18	0.0065		1	
1000	3693	47	0.0128	[a]	2	
2000	4361	111	0.0250	1		
3000	2663	106	0.0398	1	2	
5000	5666	401	0.0708	5	2	3
10,000	3456	763	0.221	11	3	

Number of breaks induced by caffeine per chromosome. Chromosome analysis 24 hr after 1-hr exposure. Reproduced from Ref. 26.

[a] 2 cells with 10 translocations.

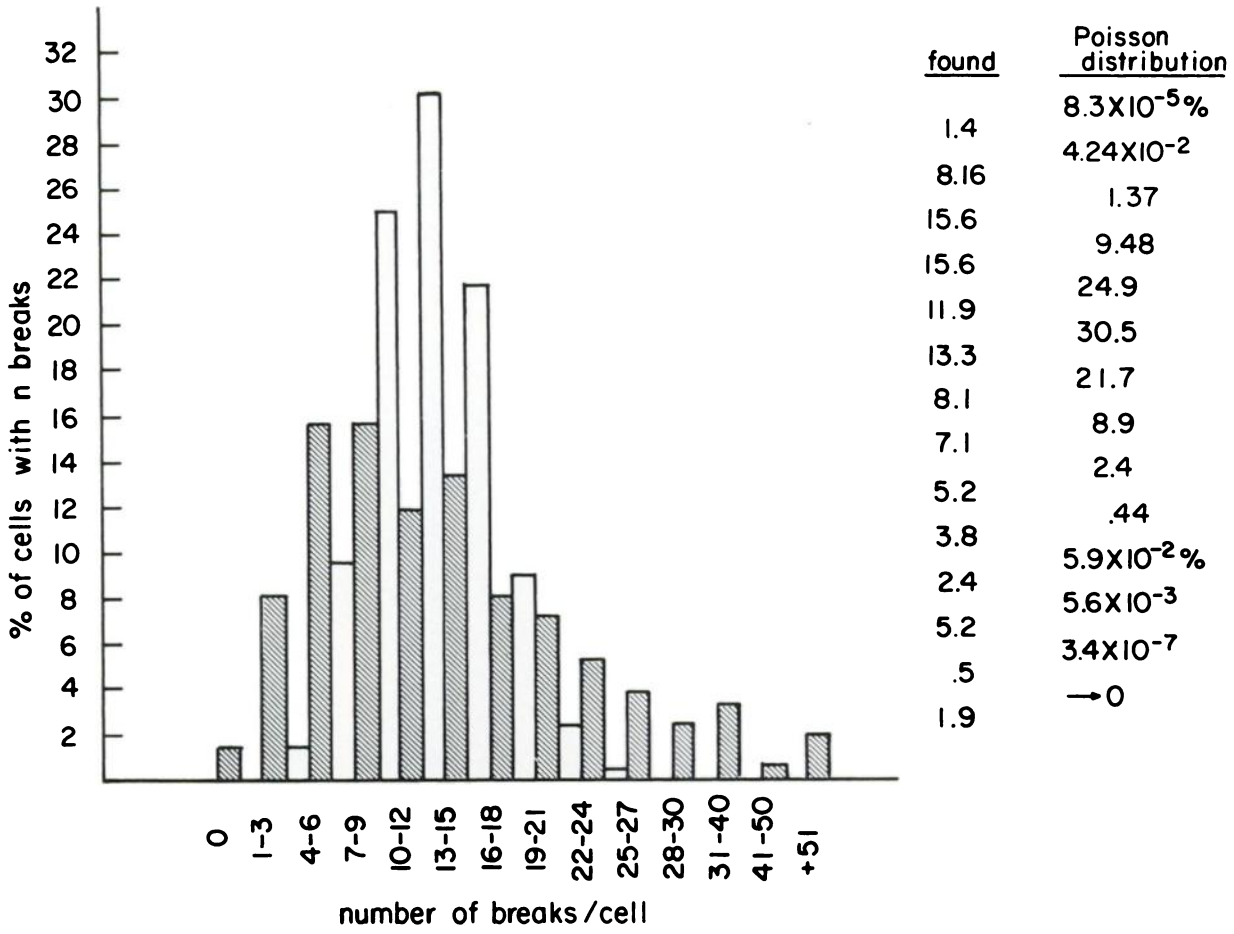

Chart 5. Number of breaks/cell using 1% caffeine, ☐, found; ▨, expected.

response is linear if we exclude these cells with an abnormally high number of breaks even at the higher dosage (1%, 0.5% caffeine). The distribution of breaks per cell of the remaining cells corresponds now to the Poisson distribution which would be expected if caffeine-induced damage is due to a single-hit phenomenon.

Time of Action during the Cell Cycle. Caffeine even in high doses affects the cell cycle of HeLa cells so slightly that it can be neglected. To test the action of caffeine on the various stages of the cell cycle, we treated the cells with caffeine for 1.5 hours at different intervals before fixation. The results are shown in Chart 6. Apparently the caffeine-induced breakage is realized only during DNA synthesis, since treatment of cells with caffeine in the G-2 phase does not induce breakage in the first mitosis after *treatment*, but in the first mitosis after *DNA synthesis*. The average probability of breakage induction for any period of the cell cycle seems to be the same (24). This is different for breaks induced in leukocyte cultures (28). The dependency of breakage on replication suggests that caffeine-induced breaks are not closely dependent on the total time of exposure if treatment is during one cell cycle before DNA replication. We tested this

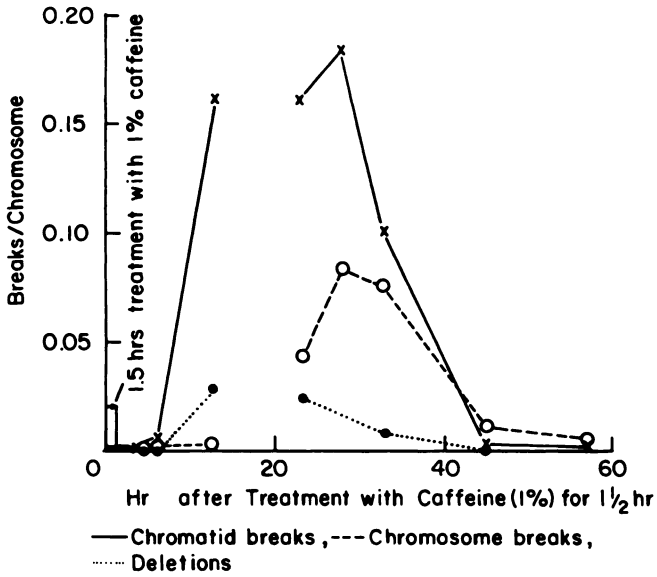

Chart 6. Breakage of chromosomes by caffeine in relation to the time of treatment during the cell cycle. HeLa cells [Ostertag (24)].

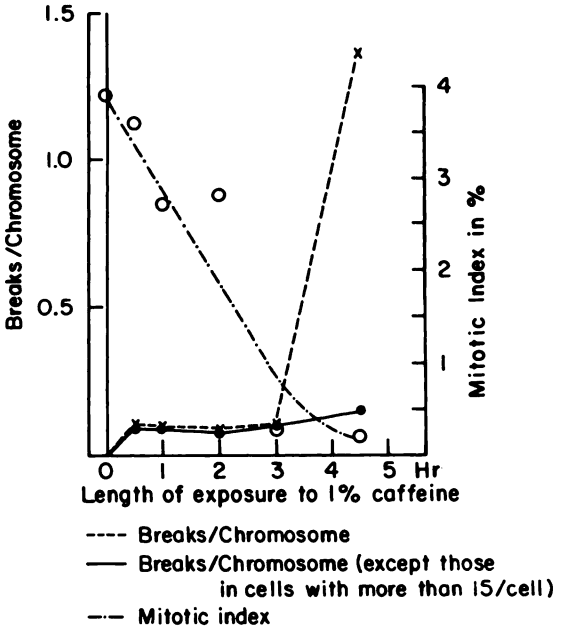

Chart 7. Dependence of caffeine-induced breakage on total time of exposure to caffeine. HeLa cells. [Ostertag (24)].

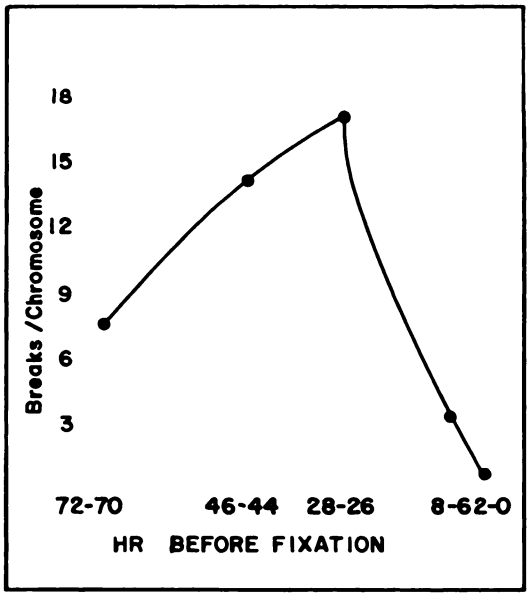

Chart 8. Breakage of chromosomes by caffeine in relation to the time of treatment during the cell cycle. Cells in leukocyte culture. (1% caffeine, 2-hr treatment).

question using 1% caffeine and varying lengths of exposure (Chart 7). The predication that caffeine-induced breakage is only dependent on dosage was shown to be true for exposure times up to four hours. After this, extensive, irreversible cell damage can be observed. This is probably the cause of the increase in the total amount of breakage after 4 hours treatment with 1% caffeine. Apparently a secondary physiologic kind of damage, probably the same as described above, occurs at high doses and longer exposure which leads to nonspecific breakage (24). Caffeine-induced breakage dependent on DNA synthesis could also be demonstrated by our results with human leukocyte cultures (28) (Chart 8). Breakage in these cells is *most frequent* if the cells are treated in middle and late G-1 phase, low in late DNA synthesis period, and *almost absent* if treated during G-2. If treatment is in early G-1, the breakage is also relatively low. This could be due to the as yet low rate of mRNA synthesis during early G-1 shortly after stimulation with phytohemagglutinin. Ts'o *et al* (36, 37) previously have shown that caffeine forms complexes with DNA, both with the coil, and to a lesser extent, with the helix DNA. DNA is probably in the coil or single-stranded form during mRNA synthesis at the site of mRNA synthesis. Razavi (34) has found single-stranded DNA by means of immunofluorescent antibody staining in lymphocyte chromosomes throughout the cell cycle. If caffeine binds mainly at the sites of mRNA synthesis, one should observe less breakage after treatment in a period with a low amount of mRNA formation. This has been found.

Chromatid Breakage in Leukocyte Cultures. Caffeine induces breakage not only in HeLa cells, but also in short term leukocyte cultures. The breakage per chromosome is closely correlated with the measured physical length of the same chromosomes if treatment is before DNA synthesis in G-1. This is no longer true if treatment is in late S phase towards the end of DNA synthesis, as can be shown by plotting the distribution of breaks. Instead there is much reduced breakage, predominantly in late replicating chromosomes (B chromosomes, and perhaps one of the X chromosomes of the female cells) (Chart 9). Presumably, the late replicating chromosomes would be underrepresented if one could also score the next mitosis, and all other chromosomes would probably be overrepresented. These results show again the correlation of DNA replication and chromosome breakage by caffeine (28). If we plot the distribution of breakage per different chromosome regions, we observe a marked deviation from expectation (28). Centromere regions show less and telomere regions more breakage than expected. This agrees with the distribution of breakage found after treatment with bromodeoxyuridine (10), but is in contrast with the spectrum of breakage after exposure of mammalian cells to actinomycin D (24, 30) and hydroxylamine (35). Actinomycin D and hydroxylamine preferentially induce breakage in the centromere regions. A correlation of the breakage spectrum to base composition of the DNA seems premature and unwarranted.

Action of Caffeine on Mice. Lyon *et al.* (15) have found no significant increase in mutation rate ("single locus test" for recessive mutations) after treatment of male or female mice with caffeine. The expected mutation rate, if the amount of breakage can be taken as a measure for gene mutation, should, however, be in the neighborhood of the natural mutation rate with the doses used by Lyon *et al.* Their data are not sufficient to exclude such a possibility. Moreover, if the mutagenic specificity of caffeine is unique, as suggested by the ability to induce breaks that have lost the capacity to rejoin, we might not find the type of mutations Lyon *et al.* were looking for. In another earlier study, Cattanach (3) showed that there is a

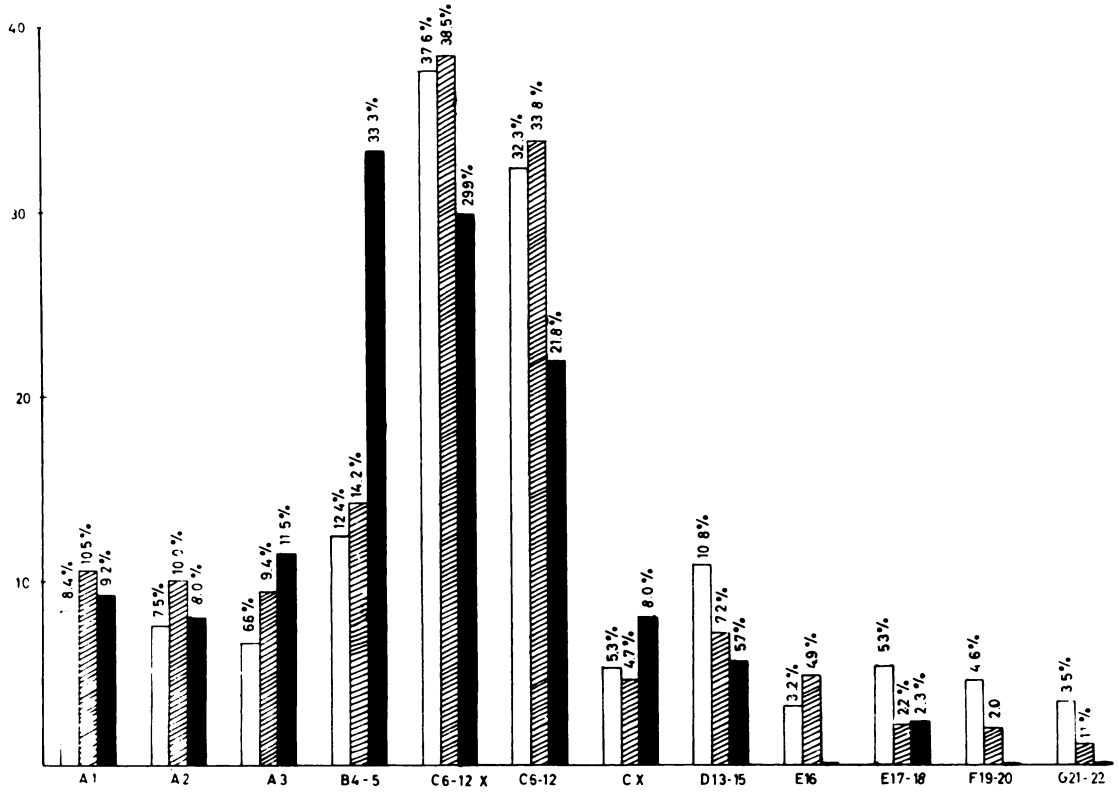

Chart 9. Distribution of caffeine-induced breaks: ☐ ,relative length of chromosomes; ▨, breakage/chromosome in % treatment in G-1; ■. breakage/chromosome in %, treatment in late S phase. Abscissa, chromosome number; ordinate, percent.

distinct decrease in fertility (which might in part be due to induced dominant lethals acting before implantation of the blastocyst) if male mice are treated with caffeine. These results were recently confirmed by us and extended by the group of Dr. Legator (unpublished results) at the FDA using rats. Even at dosage levels corresponding to 3 cups of coffee in man, there is a significant reduction of fertility if male rats are treated over a longer period. Cattanach noticed some cytologic aberrations in mice after caffeine treatment, but generally he concluded, "Without assumptions *ad hoc*, such as the production of breaks without rejoining ability, it is indeed difficult to see how a chemical that produces dominant lethality through chromosome breakage should fail to produce at least some viable rearrangements." We have shown that this is indeed the case; caffeine does induce breaks without ability to rejoin. We therefore reinvestigated the possibility of caffeine as a mutagen in mice. Since caffeine-induced breaks are only realized during replication or repair [according to our results and results obtained with bacteria (14)], we could not expect any pronounced effect on female germ cells since, in those, DNA replication is terminated after birth. We therefore exposed C57BL male mice to caffeine in their drinking water at various concentrations ranging from 0.025 to 0.5%. To get an accumulation of genetic damage, we treated for a longer period of time—on the average, 100 and 140 days. With each replication the caffeine-induced damage should increase by a constant factor. The treated males were then mated to C3H females, usually after exposing the treated males for three weeks or more to drinking water free of caffeine to eliminate any possible physiologic effect on the developing sperm cells. The preliminary results (Table 2) show that caffeine does induce dominant lethal mutations as indicated by (*a*) preimplantation losses and (*b*) resorptions, inviable embryos that can be seen after sacrificing and dissecting the pregnant female before term. If both types of dominant lethals are pooled, we get an initially linear increase of dominant lethals with caffeine dose (Chart 10).[3] The increase is less than linear at higher doses even if the results are corrected for doubly lethal hits using the Poisson distribution. The data are, however, not sufficient to establish a good dosage relationship. We conclude that caffeine is mutagenic for mammals not only *in vitro* but also *in vivo* (Kuhlmann and Ostertag, unpublished results). At the present time, Röhrborn at the Institute for Human Genetics in Heidelberg is checking whether dominant lethals in mice are induced at dosage levels corresponding to those to which humans are exposed.[4]

Action of Caffeine on *Drosophila melanogaster*. There are two conflicting reports on the possible mutagenic action of

[3] In each of the caffeine exposed series we have found a clustering of "dominant lethals." There is still a significant increase in dominant lethals if these clusterings are excluded.

[4] Dr. Röhrborn has now found some effects of caffeine using inbred strains of mice but no or little if heterozygous males were used (personal communication).

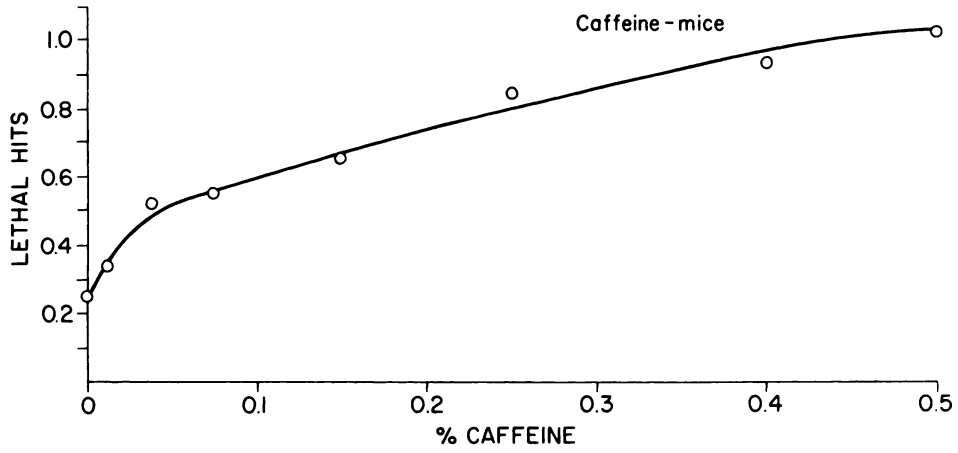

Chart 10. Dominant lethals induced by caffeine in mice. Correction applied, Poisson distribution.

caffeine on Drosophila in the earlier literature. Andrew (2) reported a significant increase of recessive lethal mutations after exposure of male Drosophila to caffeine. Yanders and Seaton (40) were unable to reproduce these results. They observed only a very slight, if any, effect of caffeine. This increase would be somewhat higher if they had included a cluster of mutations in the caffeine-treated sample. This correction is not statistically proper. In view of these results we decided to reinvestigate this question. We exposed the female larvae to 1% caffeine in a mixture of sugar, yeast, and water for one hour. They were then returned to their normal milieu, where they were allowed to hatch. We were able to observe a change in sex ratio (sex ratio is here defined as % of males in the total number of flies) in favor of the females. That is, more male than female larvae died after caffeine exposure. In the control we had 52.6% males, and in the caffeine-treated sample, 47.1% males. The average survival for the animals exposed to caffeine was 60.6%. The shift of sex ratio in favor of the females is highly significant ($P = 0.01$). Previously Oster (21), Ostertag and Muller (31), and Ostertag (23) have shown, for X-rays as well as for chemical mutagens (23), that this differential somatic mortality is due to genetic damage induced by chromosome breakage. Loss of the X chromosome in male cells in larvae presumably leads to cell death, whereas loss of one of the two X chromosomes in female cells can be tolerated. The same differential mortality, if larvae are treated with X-rays, also holds for the time after hatching. That is, X-rays induce premature aging. Premature aging can be traced back to loss of chromosomes in somatic cells (23; unpublished data). It is quite likely that caffeine induces premature aging as well. If the treated females are crossed to suitable males that are untreated, we find an increase of XO males due to chromosome loss in the progeny (F_1). The increase is in one cross from 0.15% of the controls to 0.50% of the treated animals; in a different cross, from 0.34% to 78%. There is no increase, and, in fact, possibly a decrease, in nondisjunction. The sex ratio is also changed in the progeny in favor of the females. This is an indication that caffeine does induce dominant sex-linked lethals. These results thus clearly demonstrate a significant increase in chromosome loss via chromosome breakage due to caffeine in Drosophila (29). We also tested whether caffeine induces sex-linked recessive lethals.

Table 2

Caffeine concentration[a] (%)	Matings	Pregnant animals	Corpora lutea[b]	Implantation	Living embryos	Preimplantation losses		Implant losses		Total loss (%)
						No.	%[c]	No.	%[d]	
0.025	10	8	54	41	35	13	24	6	14	35
0.05	11	10	82	55	44	27	34	11	20	46
0.1	16	14	120	88	74	32	27	14	16	38
0.2	15	8	68	36	29	32	47	7	19	57
0.3	16	11	83	56	36	27	33	20	36	57
0.5	13	10	74	41	27	33	43	14	34	64
0.025–0.5	81	61	481	317	245	164	34	72	22	49
Contr.	11	8	64	52	50	12	19	2	4	22

Dominant lethals induced by caffeine in mice.

[a]Treated for 100 days; 0.2 and 0.4% for 140 days.

[b]When the Corpora lutea could not be counted unambiguously, the average number 8 (377:47) has been used.

[c]% of total number of Corpora lutea.

[d]% of total number of implantations.

For this purpose we crossed individual F_1 females carrying one treated and marked and one untreated chromosome to males with a normal X chromosome. In the progeny (F_2) we counted those crosses with only one type of males hatching (recessive lethal for the chromosome not represented in the progeny). There is a significant increase in sex-linked recessive lethals from 0.32% of the controls to 2.48% for the caffeine-treated series (Table 3). Our results with Drosophila indicate that caffeine induces (*a*) somatic damage (and premature aging), (*b*) chromosome breakage, (*c*) dominant lethality, and (*d*) recessive lethal mutations (29). Similar results with male flies being treated with caffeine have now been reported by Mittler (16). There are some minor discrepancies between his and our results, but they need not be discussed.

Table 3

	Chromosomes tested	Sex-linked recessive lethals		
	Σ	Σ	%	P
Control	4,420	14	0.32	1
Mitomycin C	1,066	13	1.22	0.0004
Caffeine	774	19	2.48	$<10^{-10}$
Actinomycin D	408	1	0.25	0.82

Sex-linked recessive lethals, cross 2 (F_2). Reproduced from Ref. 29.

Discussion and Summary

Summarizing our main results and those of others working with caffeine as a mutagen [and both ethoxy-caffeine, (Ostertag, unpublished) and theophylline, which is one-tenth as effective as caffeine] we can conclude:

(*a*) Caffeine is mutagenic in Drosophila. It induces dominant lethals in mice if male mice are treated chronically.

(*b*) It induces chromatid breakage in human cells *in vitro*.

(*c*) The breaks do not tend to rejoin. Rearrangements are usually absent.

(*d*) The breakage is, in lower dosage ranges, linearly dependent on dose. One would thus expect that lower doses would induce breakage (mutations) in proportion to the dose.

(*e*) The caffeine-induced breakage is realized only during DNA synthesis. However, premutations can be induced in roughly the same amount throughout the cell cycle. The breakage is not dependent on the total time of exposure to caffeine. It is therefore likely, if the same is true for caffeine induced mutations in man, that caffeine induces only minimal genetic damage in nondividing tissue. Since in women the DNA replication of germinal cells is terminated with birth, almost no genetic damage would be induced in women after birth. Caffeine-induced mutagenic damage, however, could accumulate in men.

(*f*) Caffeine induces somatic damage due to chromosome breakage and loss and probably induces premature aging in Drosophila.

(*g*) Caffeine inhibits DNA dark repair as evidenced by the interference with the repair of UV-induced damage in mouse L cells (33), by the enhancement of X-ray-induced chromosome damage in leukocyte cultures (C. Jacobson, personal communication), and by the inhibition of the mitomycin C-induced increase in DNA polymerase activity in human cell cultures (39). Oxygen enhancement of caffeine-induced chromosome breakage as found in ascites tumor S_2 sarcoma in mice by Adler and Schöneich (1) can perhaps also be explained on that basis.

We would like to discuss a tentative model of the action of caffeine on a molecular basis. The model would explain both effects on repair mechanisms and induction of mutations by caffeine itself. We assume that caffeine forms complexes with DNA *in vivo* as it does *in vitro* (36, 37). This complex binding leads to inhibition in the repair of previously nicked DNA. If during replication there is only one DNA polymerase adding nucleotides in 5′–3′ direction on the new strand that is being replicated (13), we are forced to conclude either that (*a*) only one strand is synthesized in one piece in 5′–3′ direction while the other is synthesized piecewise also in 5′–3′ direction but in the opposite direction to the first strand in relation to the axis of the DNA, and only from the place of replication of the first strand to the already incompletely replicated piece of the second strand, or (*b*) that both newly synthesized strands are synthesized in small pieces. In this latter case the DNA replication would jump from the one strand to the other and would work its way backward on the other strand. The replication would then always proceed in small pieces in 5′–3′ direction which would have to be joined by a DNA ligase as occurs during repair of nicked DNA. Evidence for a piecewise replication of DNA has recently been obtained by Okazaki *et al.* (19, 20). Their results favor the piecewise replication of both strands. During both repair and normal replication, a joining of nicked or unconnected DNA strands would be necessary, which might be inhibited by caffeine. There are several possible ways that caffeine could inhibit such a process, e.g., by reacting with the joining enzyme, or by being incorporated terminally into the DNA. If this is the action of caffeine, the two aspects of caffeine action, inhibition of dark repair, and mutations realized only during replication could be due to the same basic mechanism. If the possible genetic damage by caffeine in man is estimated using the results of our experiments with human cells in culture and *Drosophila*, and assuming that caffeine is distributed evenly throughout the body, which seems to be true for the testes in men and for the germ cells of the embryo (8), we obtain a calculated total amount of genetic damage due to caffeine in the neighborhood of the natural mutation rate. One cup of coffee would then be equivalent to 0.01 R X-irradiation in men but only a fraction (1/26) of that in women (24). There is thus a strong likelihood that caffeine may prove to be one of the most dangerous mutagens in man. Further experiments using caffeine with other mammals are now in progress at several places and might lead to a more certain conclusion whether caffeine is really mutagenic in man. Data on the sex ratio of the F_2 generation in man following consumption of caffeine or theobromine in the grandparental generation should also be sought. Furthermore, experiments should be conducted to examine whether caffeine accelerates aging, induces malignant transformations, or acts as a cocarcinogen by inhibiting repair processes.

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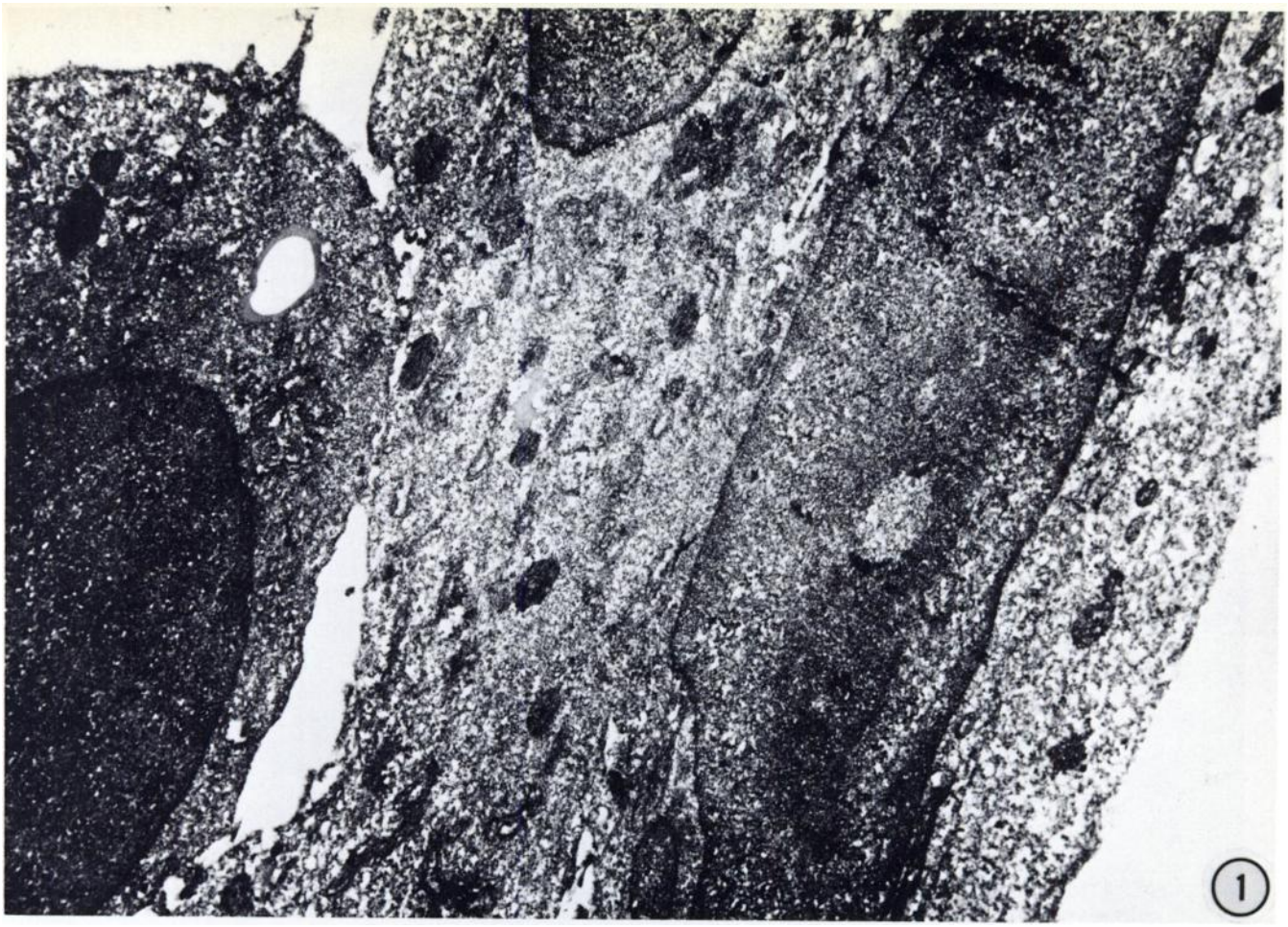
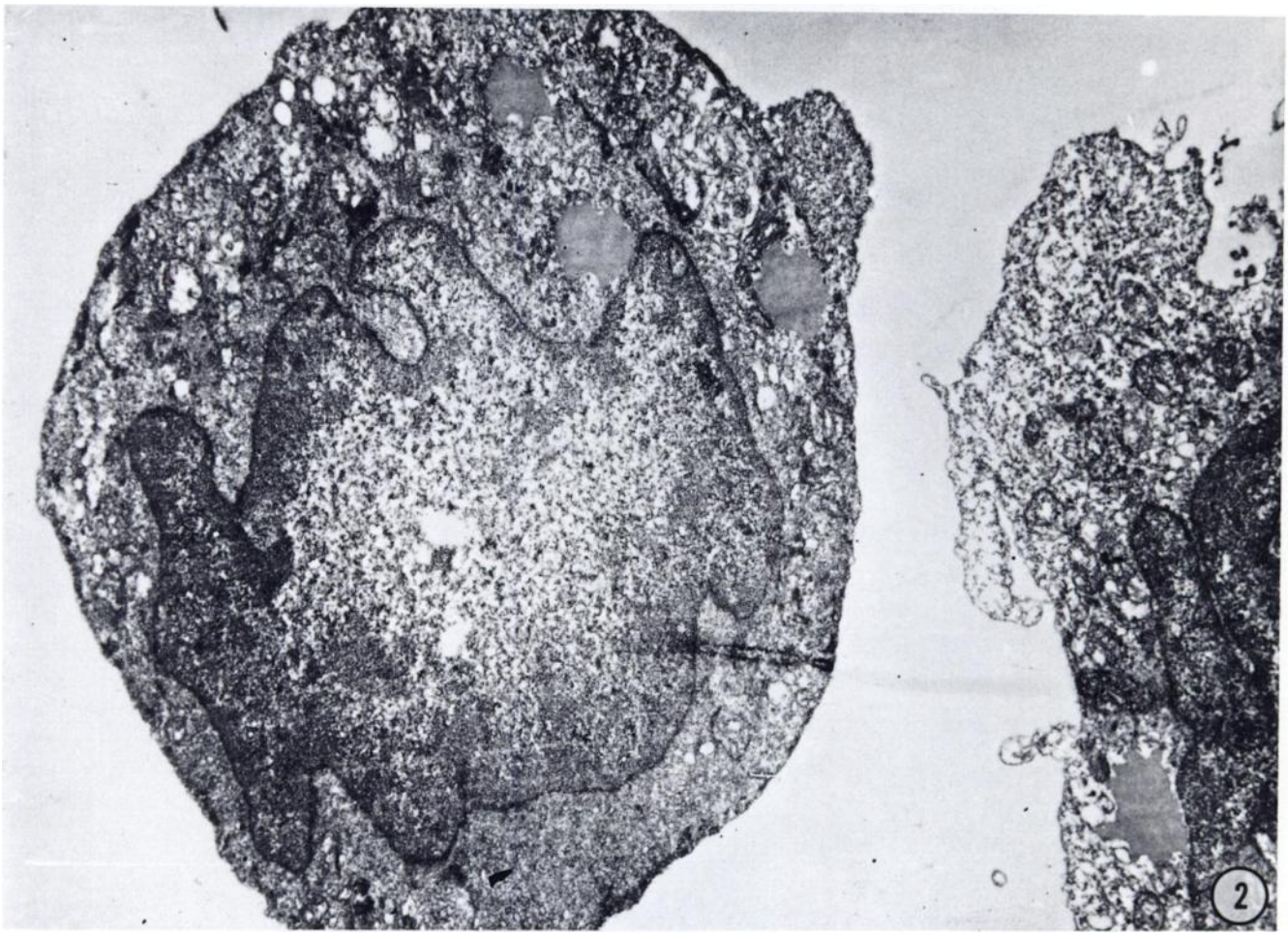
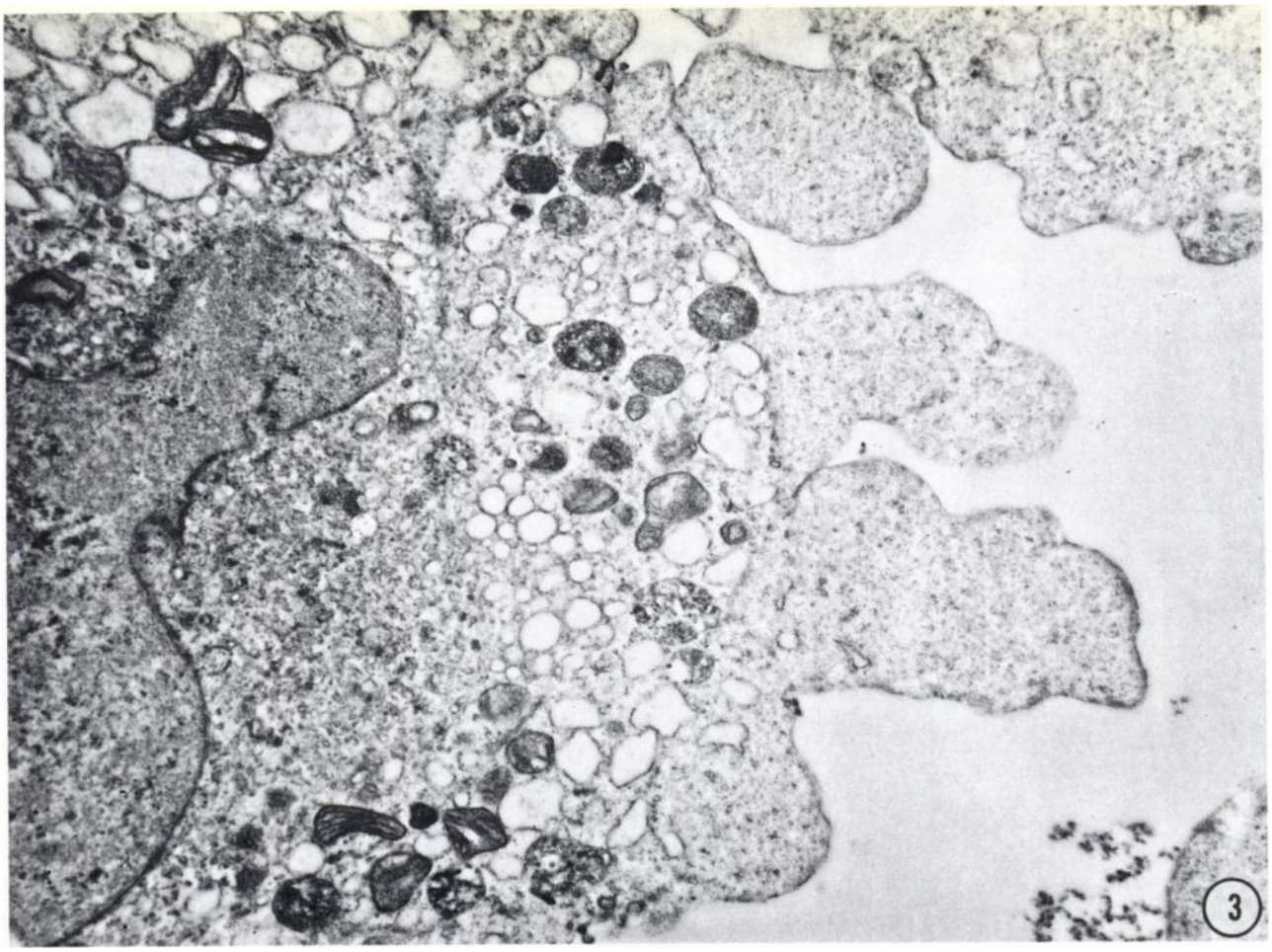
Fig. 1. HeLa cells untreated. X 18,600.
Fig. 2. HeLa cells. Treated with 1% caffeine. Cells start to round up. Beginning nuclear segmentation. × 18,000.
Fig. 3. HeLa cells. 1% caffeine. Expulsion of nuclear material.
Fig. 4. HeLa cells, 1% caffeine, 12 hr. Nuclear fragmentation X 18,000.
Fig. 5. HeLa cell, 1% caffeine, 12 hr. Cell and nucleus completely fragmented X 20,000.
Fig. 6. Nuclear fragments as seen outside the cells are no longer incorporating thymidine-^{3}H. Normal amount of label inside the cells. × 6600.
Fig. 7. HeLa cells, 1% caffeine. DNA synthesis perinuclear (labeled with thymidine-^{3}H). X 18,400.
Fig. 8. HeLa cells, control. Label is found throughout the nucleus X 18,800.
Fig. 9. Caffeine-induced breaks in leukocyte cultures. Treatment (2 hr, 1% caffeine) during G-1 phase. (Reproduced from Ref. 28)
Fig. 10. Caffeine-induced breaks in leukocyte cultures. Treatment (2 hr, 1% caffeine) during late S-phase. (Reproduced from Ref. 28)



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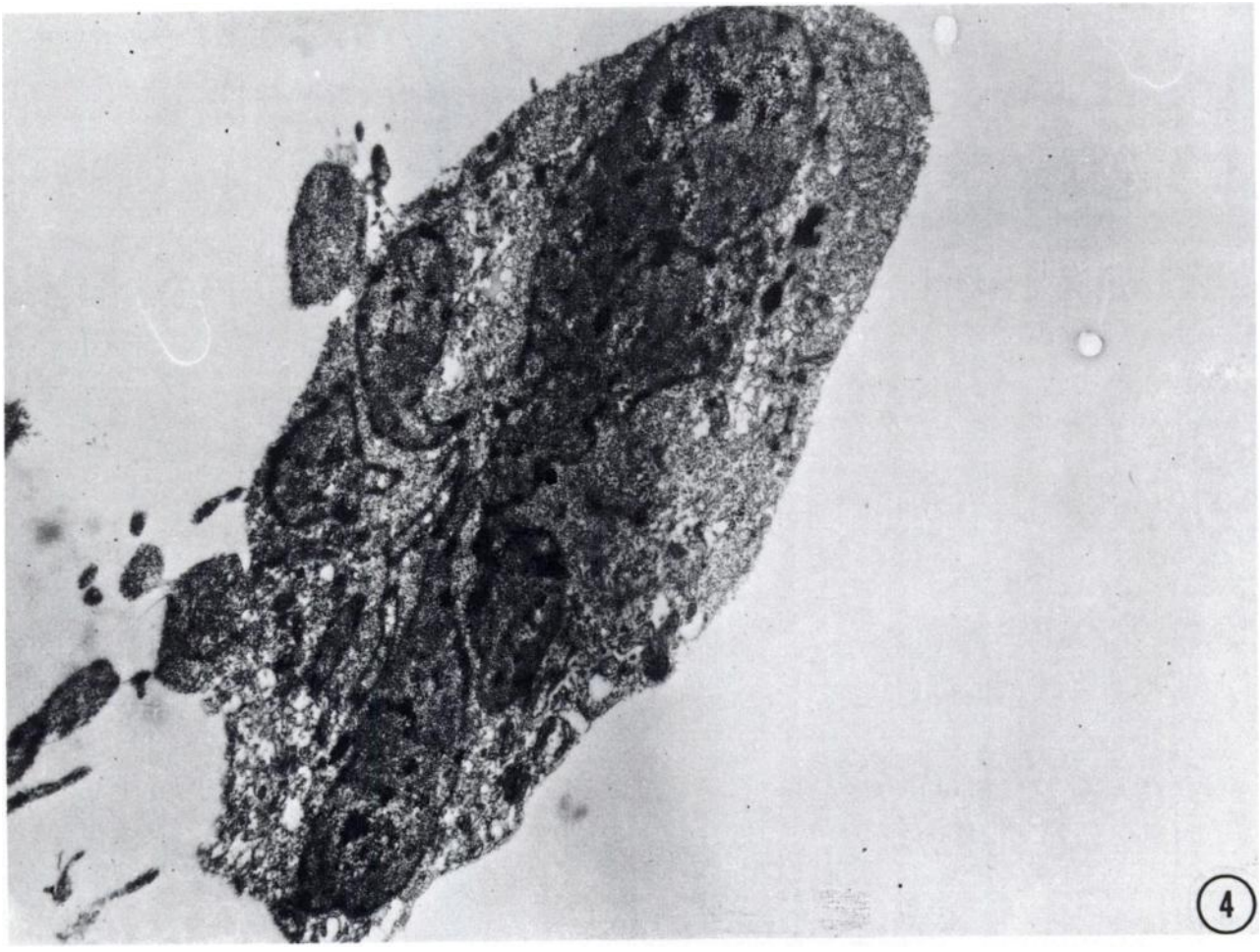
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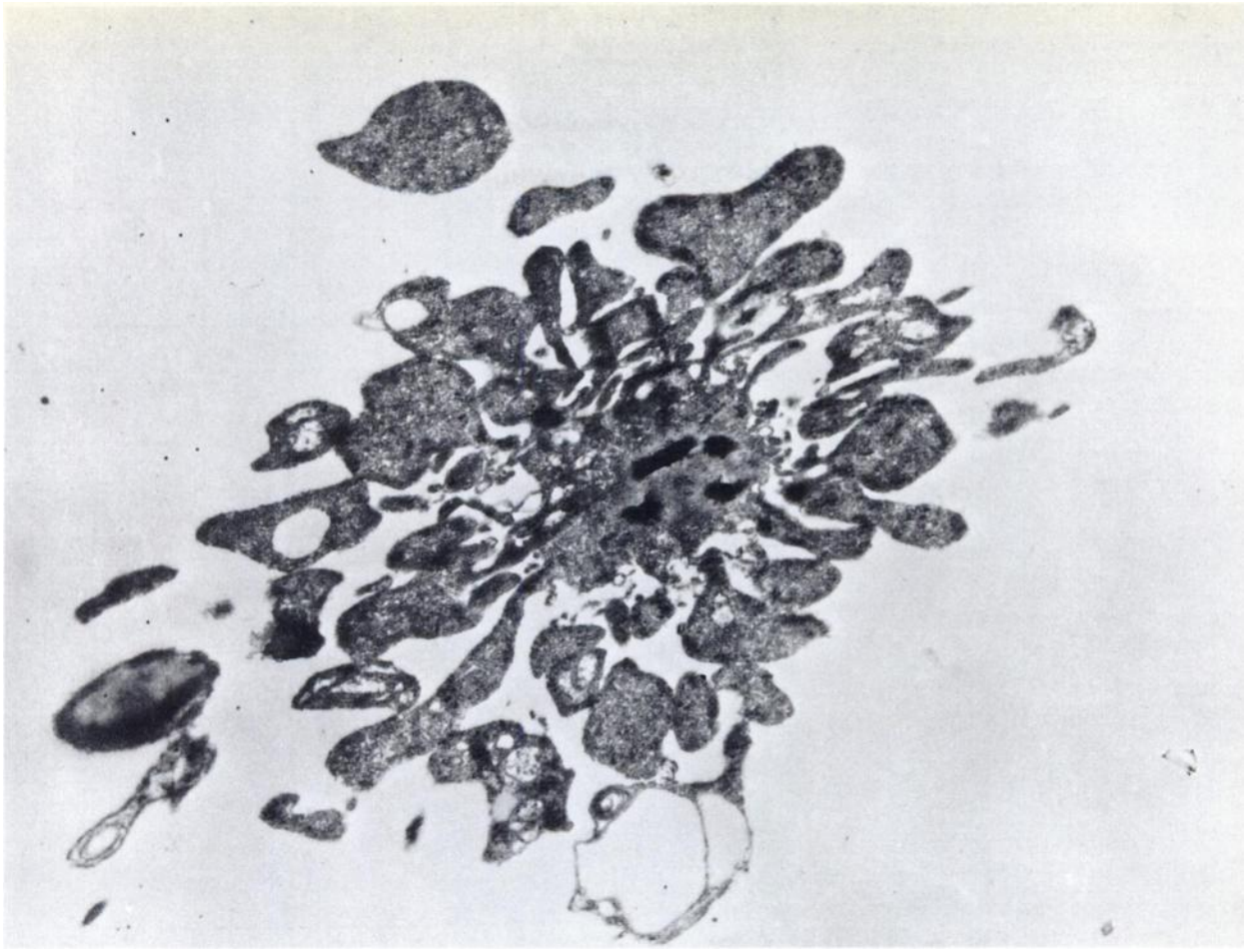

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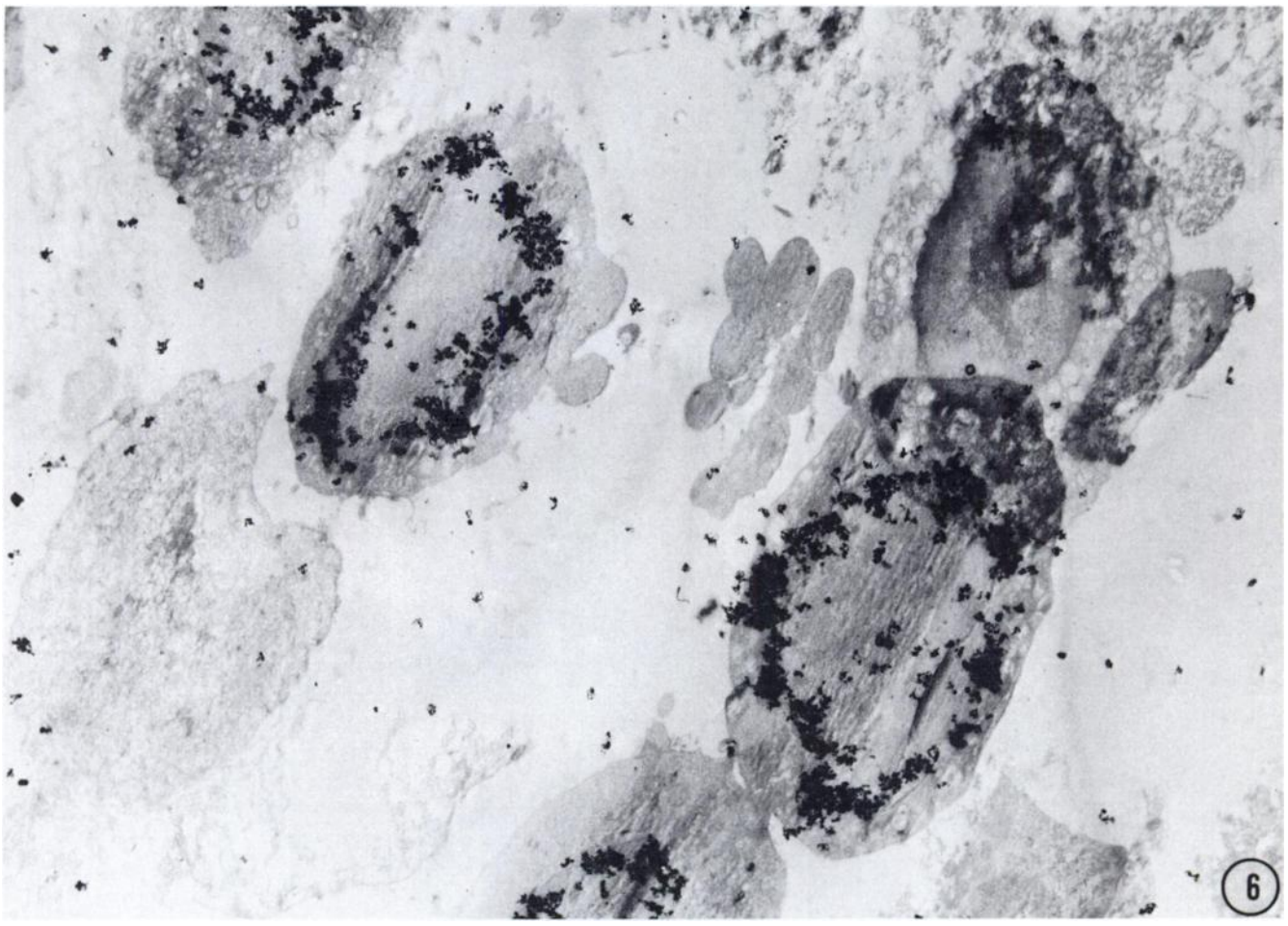

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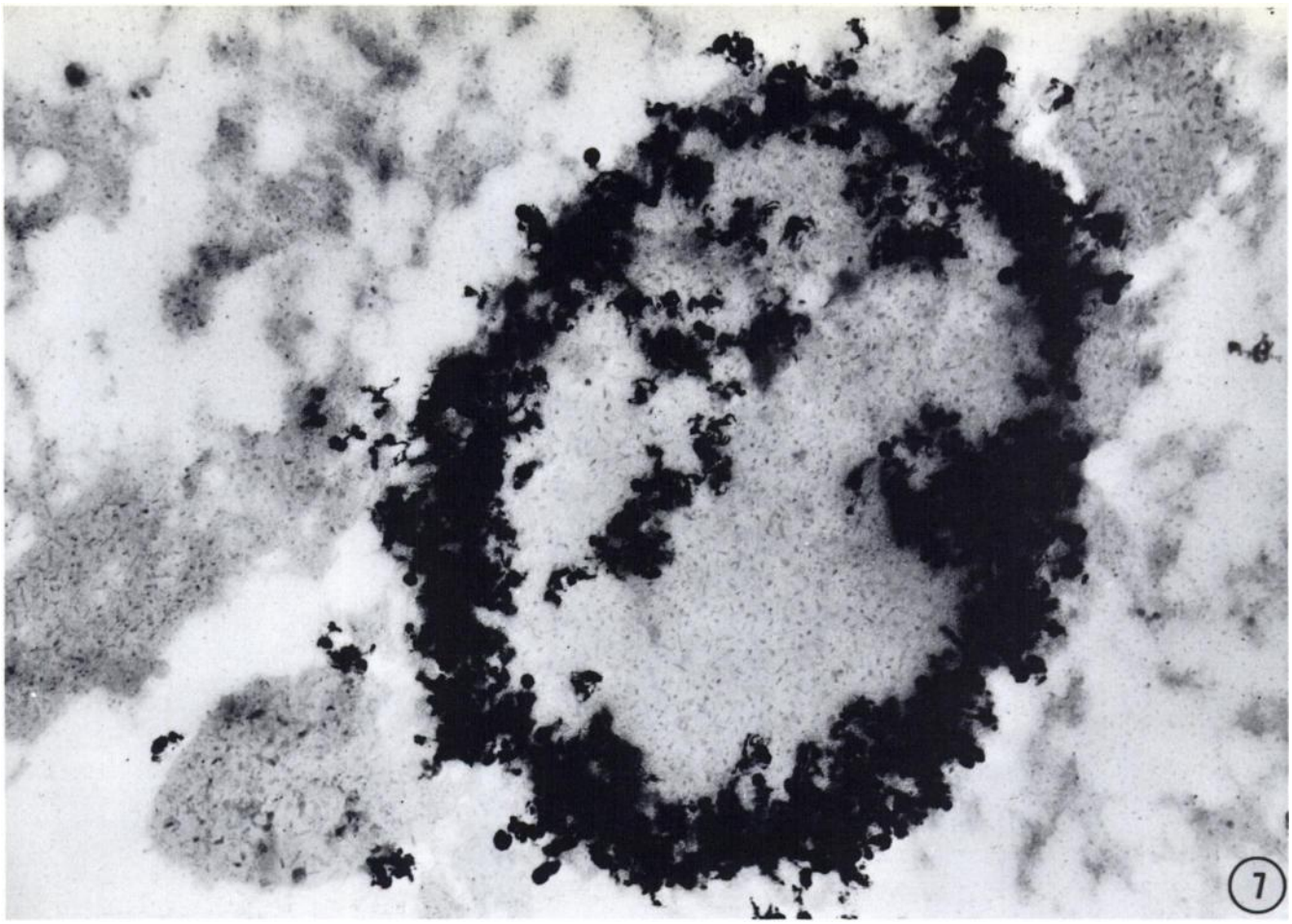
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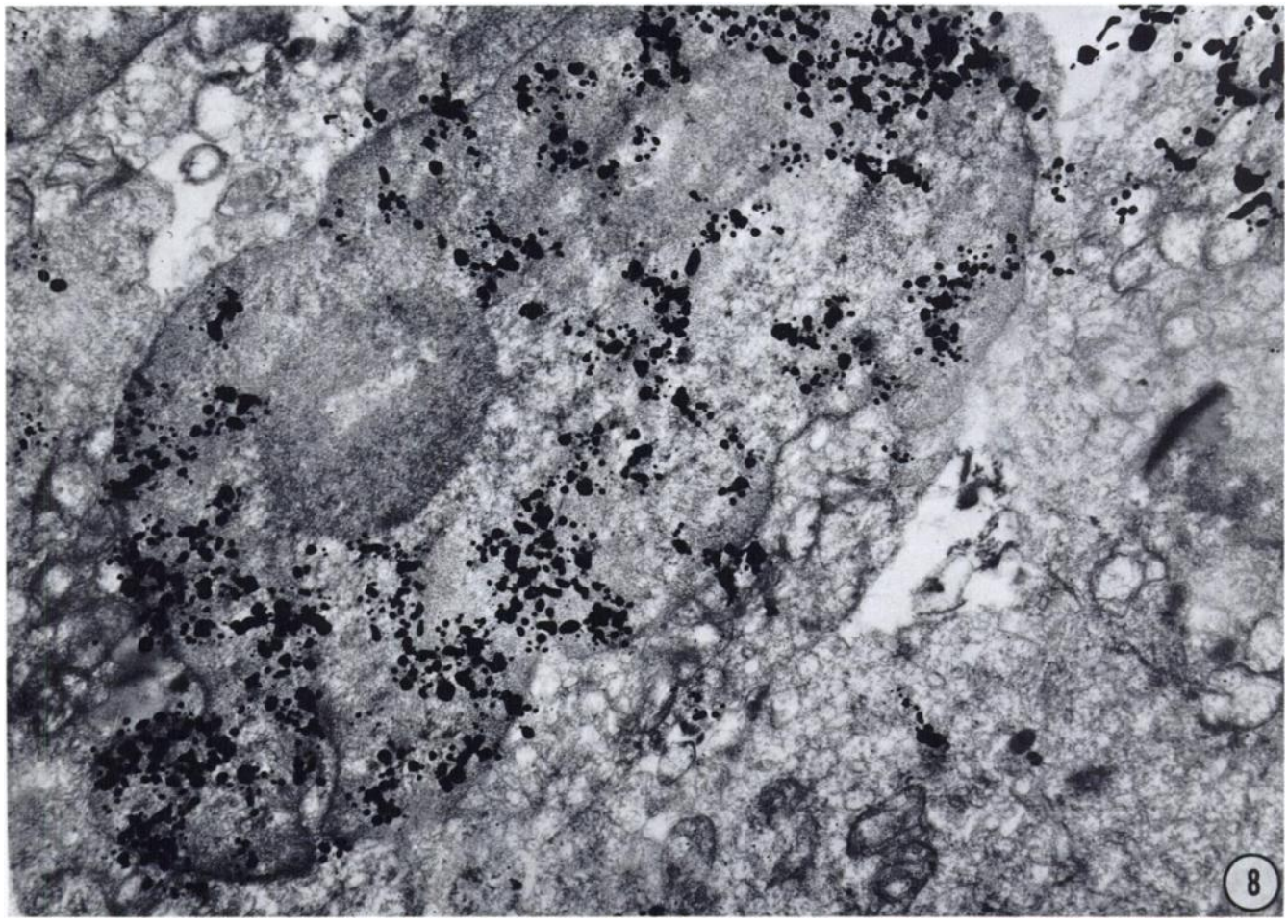
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