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Mitosis in Mammalian Cells during Exposure to Anesthetics

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The effects of methoxyflurane, trichloroethylene, chloroform, halothane and diethyl ether on the division of Chinese hamster fibroblasts in spinner culture have been studied. All agents caused dose-dependent inhibition of cell multiplication. Halothane increased the cell cycle time roughly in accordance with its effect on multiplication rate. There was no evidence that mitosis was greatly prolonged, and only small numbers of "c-metaphases" were seen. However, exposure to halothane resulted in a marked and rapid reduction in the prophase count, suggesting prolongation of G_2 (the post-synthetic phase). Cine-photomicrography showed frequent delay in division of cytoplasm at mitosis, and many binucleate cells were seen. (Key words: Anesthetics, volatile, mitosis; Mitosis, anesthetic, volatile.)

IT HAS LONG BEEN KNOWN that anesthetics inhibit cell growth and division of many species, but until comparatively recently the subject was of only academic interest. The first clinical observation of the effect was the finding that prolonged administration of nitrous oxide caused depression of cell growth in bone marrow.[1] Subsequently an attempt was made to treat leukemia with nitrous oxide, and transient remissions were obtained.[2] Interference with some aspect of cell division may be involved in the observation of the teratogenic effects of clinical concentrations of nitrous oxide in the rat[3] and of cyclopropane in the chick embryo.[4]

Several papers have shown recently that staff exposed to anesthetics in operating theaters were subject to an increased incidence of fetal malformations, miscarriages, and certain forms of malignancy.[5–8] There is at present no direct evidence that these effects on dividing cells are caused by the inhalation of the trace concentrations of anesthetics known to contaminate operating theater atmospheres. However, Corbett *et al.*[9] have shown an increased rate of fetal resorption in pregnant rats exposed to 1,000 parts per million of nitrous oxide. In contrast, Bruce[10] found no such effect with 16 parts per million of halothane.

Although the inhibition of cell division has been demonstrated for many anesthetics and for several types of cells, the dose–response relationship has seldom been quantified, and the mechanisms by which anesthetics interfere with division and growth remain obscure. It seems likely that the predominant effect in plant species (*e.g.*, *Vicia faba* and *Pisum sativum*) is by a colchicine-like action on the microtubules of the mitotic spindle, resulting in an arrest in metaphase and an appearance similar to the so-called "c-mitosis."[11,12] Dispersal of the mitotic spindle by 2 per cent halothane has been observed in fertilized eggs of *Echinus*.[13]

There is evidence that growth and division of a number of different types of mammalian cell are inhibited by concentrations of anesthetics not greatly different from those that affect plant cells. However, it is not known whether the mechanisms of action differ from those observed in plants. There have been no published reports of a colchicine-type of metaphase arrest when dividing mammalian cells were exposed to anesthetics. On the other hand, there is evidence of interference with synthesis of deoxyribonucleic acid (DNA).[14,15]

Fink and Kenny[16] reported dose–response curves for inhibition of growth of mouse heteroploid cells by various anesthetics during four days' exposure. From their data, values for 50 per cent inhibition may be inferred, and these are referred to below (fig. 11). In the same study, mouse Sarcoma I cells were far more sensitive to halothane. Jackson[17] has also reported dose–response relationships for inhibition of growth of mouse hepatoma cells by halothane, but

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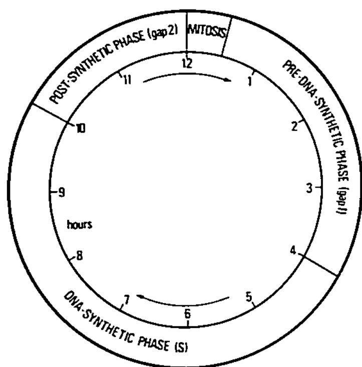

FIG. 1. Normal cell cycle of unanesthetized Chinese hamster fibroblasts. These times relate to a stirred suspension and are slightly shorter when the cells are grown in monolayer on glass or plastic.

other studies of inhibition of growth of mammalian cells do not provide data that permit calculation of 50 per cent effective concentrations.

In the present study we have established dose–response curves for inhibition of cell multiplication, using five different anesthetics and thus permitting calculation of their relative potencies. We also investigated the sensitivity of the cells to anesthetics during different phases of the cell cycle. We found the duration of cell cycle time from time-lapse cine-photomicrography and observed many abnormalities of mitosis. Throughout the study we used a line of Chinese hamster fibroblasts which has been grown in culture since 1958. This was maintained as a pure line by growing up stock from single cells on several occasions. Although these cells are undoubtedly abnormal, they are not neoplastic and they provide a model system that is stable and gives repeatable results.

Methods

Cells and Medium

The Chinese hamster fibroblast cells were originally derived from lung tissue and contain 22 chromosomes. The cells were grown as a monolayer either in glass bottles or in plastric Petri dishes specially treated for tissue culture. They were also grown as a suspension of virtually single cells in flasks stirred with a suspended or a detached rotating magnet (spinner culture). Under our conditions these cells have a doubling time of 9–12 hours, which is subdivided as shown in figure 1.

Eagle's minimum essential medium[18] with added antibiotics and 15 per cent fetal calf serum was used for growth of cells in monolayer. Spinner cultures were grown in modified Eagle's medium with added antibiotics and 7 per cent fetal calf serum. Both these media contain glucose at 1 g/l (5.6 mmol/l). Since this medium contains sodium bicarbonate as a buffer, it was necessary to keep all unsealed cultures in an atmosphere of 5 per cent carbon dioxide in air.

Exposure to Anesthetics

All controls were equilibrated with 5 per cent carbon dioxide in air. Treated preparations were equilibrated with the same carrier gas, to which known concentrations of anesthetics were added. A range of vaporizers was used to dispense methoxyflurane, trichloroethylene, halothane, chloroform, and diethyl ether. The carrier gas flow rates were always above the manufacturers' stated minimum flow rates, and surplus effluent was discharged to atmosphere. The outputs from the vaporizers as well as the effluents from the cellular preparations were always monitored with a refractometer[19] which was set to zero on carrier gas. Throughout this paper concentrations of anesthetics mentioned refer to the concentration measured in the effluent from the preparation.

For spinner cultures, carrier gas with or without anesthetic was passed over the surface of 30 ml of cell suspension with cell numbers at $2.5–3.0 \times 10^5$/ml in a 100-ml Clearfit flask, maintained at 37 C. Stirring was fast enough to cause a small vortex, which assisted equilibration. It was demonstrated by spectrophotometry (wavelength 205 nm) that under these circumstances distilled water reached equilibrium with halothane in 15 minutes. In fact, the anesthetic was passed for 30 minutes, after

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which the flask was closed with a Clearfit stopper which made a gas-tight seal without the use of grease. We demonstrated by refractometry with halothane or trichloroethylene that the composition of the supernatant gas was unchanged during a period of 24 hours, showing that there was no appreciable loss due to leakage or biotransformation.

Monolayer cultures growing in Petri dishes were placed in nylon sleeves through which various gas mixtures could be passed. The sleeves were kept in a hot room at 37 C, but the carrier gas/anesthetic mixtures were prepared in an adjoining room at normal temperature and passed through a short length of nylon tubing into the hot room. The effluent was returned to the outside laboratory for monitoring and exhaust. The sleeves were mounted on a platform that could be rocked through a small arc at a frequency of 15 cycles/min. A Petri dish could be removed from the sleeve without changing the gaseous environment of the remaining Petri dishes in the same sleeve.

For prolonged exposures (6–16 hours), Petri dishes were placed in a desiccator, which was flushed with vapor and then sealed. At the end of the exposure period, before opening the desiccator, the halothane concentration was measured by refractometry. The desiccator was opened only once in these experiments, which took place at 37 C.

Effect on Cell Count

Shortly before an experiment, Chinese hamster cells were taken from a spinner culture in logarithmic phase and diluted with medium to 2.5–3.0 × 10^5 cells/ml. Cells were counted, exposed to various concentrations of anesthetic or carrier gas (see above), sealed up, and counted again after 24 hours. Unstained cells were counted in a hemocytometer. This method was preferred to the use of a Coulter counter, as it permits exclusion of dead cells and debris and cells in pairs or clumps can be correctly recorded.

Growth rate was expressed as the reciprocal of doubling time, and the effect of the anesthetic upon it was calculated as the ratio of treated to control growth rates. A control flask was included in each experiment.

In order to fit sigmoid curves by least squares, probit transformations were applied to the growth rate (expressed as percentage of control) and, with appropriate weighting,

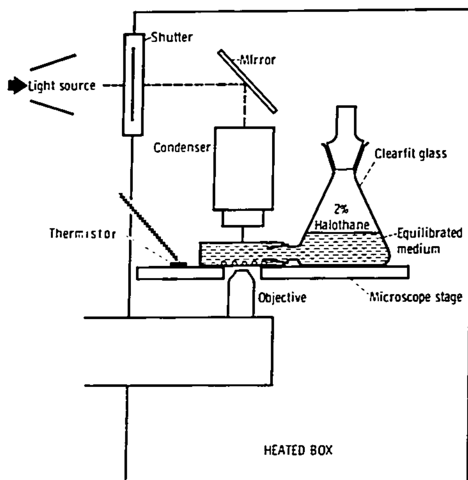

FIG. 2. Assembly for cinephotomicrography of growing cells, using an inverted microscope. The cells were filmed growing on the lower inside face of the 10 × 10 mm spectrophotometer cuvette placed on its side and acting as an extension to the 50-ml Clearfit flask, which contained medium equilibrated with anesthetic vapor. A thermistor controlled the temperature of the stage at 37 C.

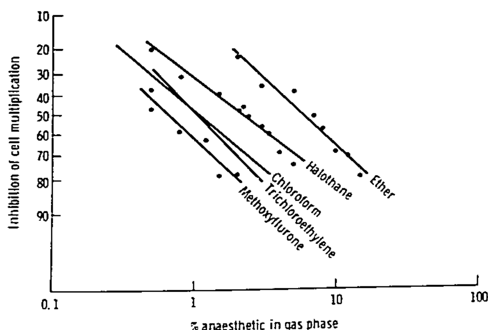

FIG. 3. Regression lines corresponding to dose–response curves for inhibition of cell multiplication with five inhalational anesthetics. The abscissa is a log scale and the ordinate is a probit scale. Individual points are plotted for methoxyflurane, halothane and diethyl ether, but omitted for reasons of clarity in the case of chloroform and trichloroethylene, since curves for these agents are almost superimposed. Values for ED_{50} are in table 1.

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regression lines were calculated against the logarithm of the concentration of the anesthetic in the gas phase. Since there were no statistically significant differences between the slopes of the five regression lines, they were recalculated on the basis of all slopes being the same and equal to the pooled slope of the five. Values for ED_{50} were calculated from both sets of regression lines and 95 per cent fiducial limits were determined.[20]

Measurement of Cell Cycle and "Rounded-up" Time

Chinese hamster cells assume a spherical shape during division and are then highly refractile as seen under the microscope. The period when they are rounded-up includes the whole of mitosis, but it is not usually possible to define the onset or completion of the phases of mitosis, although anaphase can sometimes be seen by phase-contrast microscopy. Cell separation can be clearly seen, and lasts only 2–3 minutes. We have used this end-point for determination of cell cycle time. We also recorded the time from round-up to cell division.

Measurements of time were made by time-lapse phase-contrast cine-photomicrography. The cells were grown on the optically flat surface of a 10 × 10 mm spectrophotometer cuvette, laid on its side. A 50-ml Clearfit conical flask was adapted to fit on to the cuvette, as shown in figure 2, and the whole assembly was laid on the stage of a Wild inverted microscope maintained in a hot box with temperature controlled at 37 C by a thermistor mounted on the stage. The objective was × 20 phase and eyepiece × 6. Cine-photography was with a Bolex 16-mm camera, exposures being at 1-minute intervals, maintained for 44 hours. A solenoid device restricted illumination to a period of 0.5 seconds in each minute, and this included the exposure time, which was 0.2 seconds. The film was projected with a Spectro analyzing projector and analyzed by single frames.

The cuvette–flask assembly (fig. 2) was filled with a diluted suspension of cells and put aside for 10 minutes at 37 C for cell attachment. Surplus suspension was then discarded and the assembly was refilled with 20 ml of medium. For the control film the medium in the flask and cuvette was equilibrated with carrier gas during magnetic stirring, as described above, for 30 minutes at 37 C. The flask was then sealed and the assembly transferred to the microscope. For the treated preparation the procedure was the same, except that there was 2% halothane with the carrier gas. This was checked by refractometry.

Sixteen-millimeter film did not give adequate definition for detailed examination of cell morphology. We therefore grew separate batches of cells on microscope slides in shallow medium in Petri dishes. These were exposed at different times to various concentrations of halothane in a desiccator at 37 C.

TABLE 1. Dose-response Characteristics of Anesthetics in Inhibition of Cell Multiplication*

	Methoxyflurane	Trichloroethylene	Chloroform	Halothane	Diethyl Ether
ED_{50} from calculated regression lines (fig. 3)	0.67	1.05	1.06	2.17	5.97
Slope of calculated regression lines (fig. 3)	−1.79	−1.86	−1.53	−1.39	−1.69
ED_{50} from parallel regression lines with 95 per cent fiducial limits	0.65 (0.56–0.75)	1.01 (0.89–1.15)	1.06 (0.96–1.18)	2.19 (1.96–2.44)	5.93 (5.23–6.70)

* Anesthetic concentrations as percentages of one atmosphere in the gas phase.

After 24 hours cover slips were applied and phase-constrast microphotographs were made of treated and control preparations.

Serial Changes in Mitotic Counts

Mitotic index was determined and counts of the different phases of mitosis were made at intervals during the first three hours and after 6 and 16 hours of exposure of cells to 2 per cent halothane. An inspection was also made for abnormal mitotic figures, particularly the type of metaphase arrest typical of the action of colchicine (c-mitosis). For this purpose cells at 2–3 × 10^5/dish were grown overnight on microscope slides immersed in medium in Petri dishes. After they were established in logarithmic phase they were treated with halothane in the nylon sleeve, as described above, in parallel with a second sleeve containing control cells exposed only to the carrier gas. The platform was rocked for the first 15 minutes of treatment to hasten equilibration. Dishes were removed from control and treatment sleeves ½, 1, 2, and 3 hours after starting the anesthetic. For comparison, one preparation which was not exposed to anesthetic was treated with colchicine (0.5 μg/ml) for 3 hours. After treatment slides were immediately fixed in a mixture of 3 parts absolute alcohol with 1 part of glacial acetic acid, dried, and stained with 2 per cent acetic orcein. The number of prophase, metaphase, anaphase and telophase figures in dividing cells were counted to a total of 100 on each slide and expressed as a percentage of total mitoses. Mitotic index was calculated from a count of 1,000 cells per slide. Results for treated cells were then expressed as percentages of control counts.

A separate batch of cells, exposed as above, was expanded with hypotonic saline solution and then stained as described. These preparations, both control and treated, were used for quantification of abnormal nuclei and in a search for chromosomal aberrations.

Results

Effect on Cell Multiplication Rate

Methoxyflurane, trichloroethylene, chloroform, halothane, and diethyl ether all caused dose-dependent depression of the cell multiplication rate (fig. 3). Slopes of the various dose–response curves were not significantly different, and ED_{50} values are therefore better derived from the calculated parallel regression lines (table 1). In fact, the

TABLE 2. Duration of Cell Cycle and Interval from Rounding-up to Cell Division

	Control	2 Per Cent Halothane
Cell cycle time		
Number of cells	13	12
Mean time (hours)	10.19	20.87
SD	1.86	4.17
SEM	0.52	1.20
Round-up to cell division		
Number of cells	15	22
Mean time (minutes)	26.2	36.6
SD	5.2	17.2
SEM	1.3	3.7

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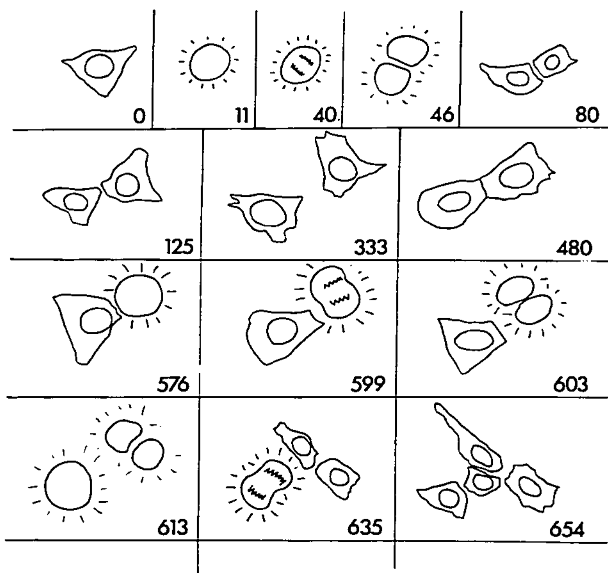

FIG. 4. Tracings from a time-lapse film showing formation of a normal clone in an unanesthetized preparation of Chinese hamster cells growing on glass. Elapsed times are in minutes.

two methods of calculation give values for ED_{50} which in no case differ by more than 0.04 per cent, which is insignificant. Depression was observed at the minimum alveolar concentration required for anesthesia, where this was used. The minimum concentration of methoxyflurane studied was 0.5 per cent, and this resulted in a growth rate of 60 per cent of control.

CELL CYCLE AND TIME BETWEEN ROUNDING-UP AND DIVISION

Mean cycle time was 10.19 hours in the control films; this was increased to a mean time of 20.87 hours in the preparation in equilibrium with 2 per cent halothane (table 2). The difference was highly significant ($P < 0.001$).

The interval between rounding-up and cell division was increased from 26.2 minutes in the control to 36.6 minutes after equilibration with 2 per cent halothane (table 2). The variability was greatly increased in the anesthetized preparation, but the change was significant ($P < 0.02$).

The formation of a control clone is shown during two generations in figure 4, with cell cycle times of 9.28 and 9.90 hours for the sister cells. Figure 5 shows single divisions of two cells exposed to 2 per cent halothane. It is probable that they were sister cells. The sequence shows relatively normal division of the nuclei followed by failure of division of the cytoplasm. In the case of the first cell to divide, cytoplasmic division never occurred, while, in the second case, the daughter cells remained joined by a thread (170 minutes)

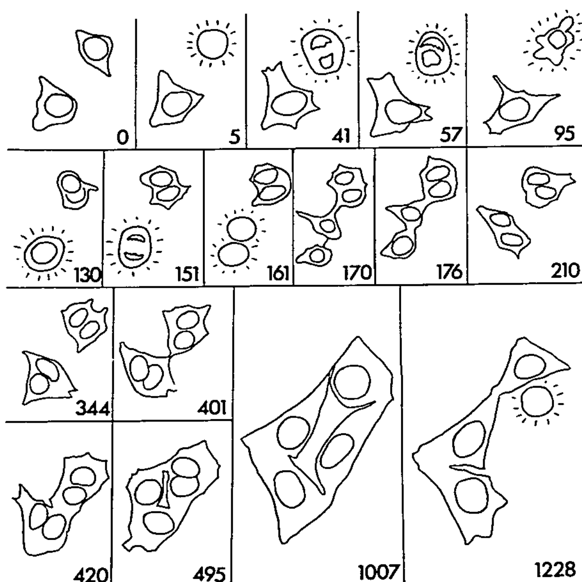

FIG. 5. Tracings from a time-lapse film showing division of two cells (probably sister cells) in equilibrium with 2 per cent halothane. Elapsed times are in minutes. See text for commentary.

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but appeared as a binucleate cell 6 minutes later. By 344 minutes there were two distinct binucleate cells, which by 420 minutes had fused to form a single cell with four nuclei, although partial separations were visible. Eventually each nucleus rounded-up for division separately, in 1,186, 1,282, 1,314 and 1,662 minutes, respectively; the first of these events is shown in figure 5 at 1,228 minutes. A complex of eight nuclei was established at 1,750 minutes.

Figure 6 shows a normal control culture growing on glass with typical appearance as seen by phase contrast microscopy. Figure 7 shows cells after 24 hours in equilibrium with 2 per cent halothane. Two multinucleate cells are clearly visible. Counts of abnormal nuclei in stained preparations also showed a marked increase in the numbers of bi- and trinucleate cells and in cells containing micronuclei after growth in equilibrium with 2 per cent halothane (table 3).

SERIAL CHANGES IN MITOTIC COUNTS

Mitotic index values fell rapidly to about 60 per cent of control and were still low after 16 hours of exposure to 2 per cent halothane (fig. 8 and table 4). This contrasted with the colchicine-treated cells, in which the mitotic index was 250 per cent of control after 3 hours.

Prophase count fell rapidly and remained depressed throughout exposure to 2 per cent halothane (fig. 9 and table 4). There was some evidence of recovery, but during the first two hours of anaesthesia the count, expressed as percentage of all cells, was only

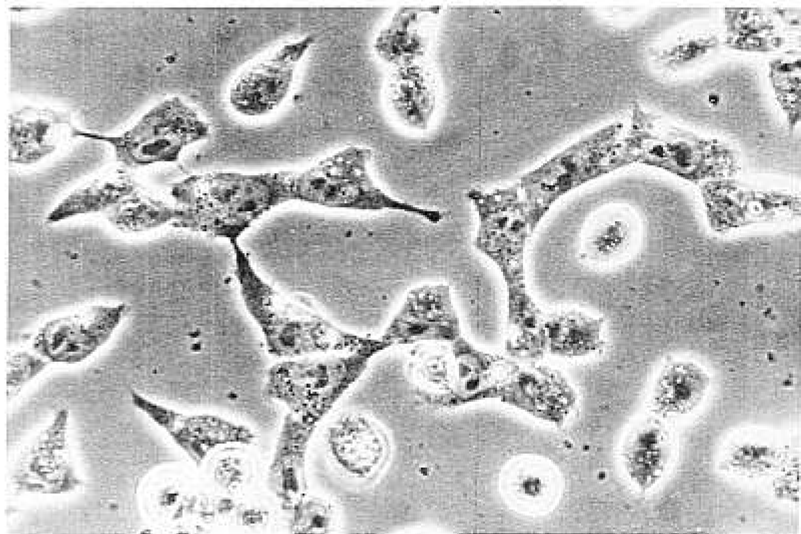

FIG. 6. Normal control culture growing on glass (phase contrast). The circular cells are rounded-up for division, and details of their internal structure cannot be seen. Other cells show the nuclear membrane and usually two nucleoli.

26 per cent of control values. Prophase counts were not greatly changed by exposure to colchicine.

Metaphase count (as percentage of cells in mitosis) was unaffected by halothane, but greatly reduced by colchicine (table 4). However, when expressed as a percentage of all cells, halothane caused a modest and sustained reduction in the count (fig. 9 and table 4).

Exposure to 2 per cent halothane resulted in small but consistent increases in the numbers of c-metaphases during the first two hours. Numbers in the control preparations were too small to express results as percentages of control, and figure 10 therefore shows actual c-metaphase counts expressed as percentages of all mitoses. Counts in pooled controls are indicated as horizontal bars. No c-metaphases were seen after 6 and 16 hours of exposure to anesthetic. These results contrasted with the hundredfold increase in c-metaphases seen after treatment with colchicine (table 4).

Anaphase and telophase counts were reduced but did not disappear in the presence of halothane (fig. 9 and table 4). In contrast, anaphase and telophase figures were markedly reduced when cells were treated with colchicine. After treatment with halothane a number of abnormal metaphases and anaphases and a few anaphase cells with bridges were seen. No damage to individual chromosomes was observed, but nuclei in which all the chromosomes were degenerating were occasionally present.

Discussion

The multiplication rate of Chinese hamster cells in logarithmic phase is depressed in a dose-dependent manner by all five anesthet-

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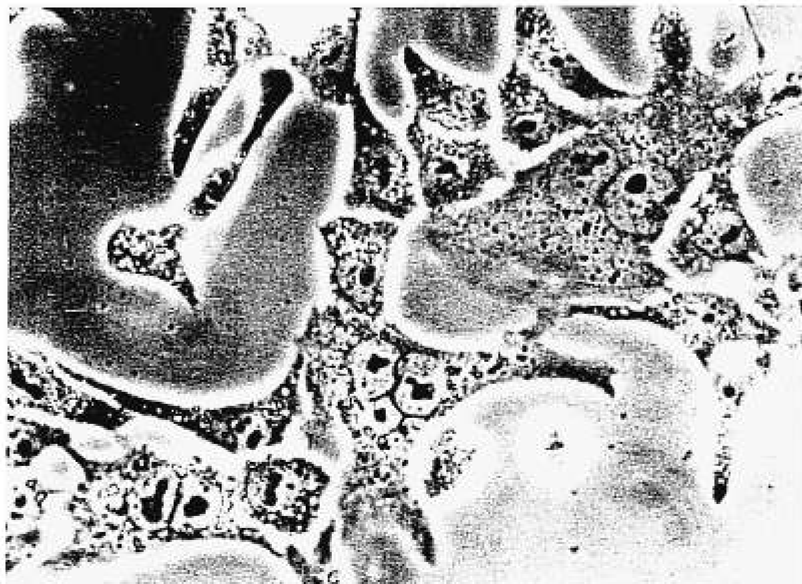

FIG. 7. Cells growing on glass in the presence of 1 per cent halothane. Two binucleate and one trinucleate cell may be seen.

ies tested. The concentrations required for 50 per cent depression of growth ranged from 1.4 to 4.2 times the minimum alveolar concentration (MAC) required for anesthesia (table 5). These values are not greatly different from those reported for mouse heteroploid cells by Fink and Kenny,[16] and for rat hepatoma cells by Jackson.[17] We are in agreement with Fink and Kenny[16] that chloroform, relative to its MAC value, is the most potent in its action on the dividing cell, and trichloroethylene the least potent. These differences may also be seen when ED_{50} values for effects on the dividing cell are

TABLE 3. Abnormal Interphase Cells Observed on Slides after 24 Hours Exposure to 2 Per Cent Halothane*

	Binucleate Cells	Trinucleate Cells	Cells with Micronuclei	Total Abnormal Cells
Control	0.31	0	0.11	0.42
SD	0.18	0	0.07	
SEM	0.07	0	0.03	
Halothane, 2 per cent	3.5	1.07	1.7	6.3
SD	0.6	0.46	0.36	
SEM	0.35	0.27	0.21	

* Values are percentages of 1,000 interphase cells.

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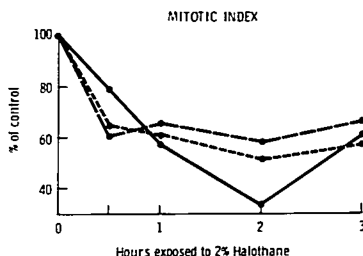

FIG. 8. Serial changes in mitotic index during 3 hours of exposure to halothane. The individual results of three experiments are presented as the precentages of values obtained in simultaneous controls.

plotted against the oil–gas partition coefficients for olive oil at 37 C. In contrast to the strong correlation with MAC, there are highly significant differences in the correlations with ED_{50} for cell multiplication (fig. 11).

Table 5 also shows the values for 50 per cent depression of colony forming ability (CFA)[22] for the same line of cells. Trichloroethylene again appears to be relatively ineffective in the reduction of cell survival. ED_{50} values for cell division and for

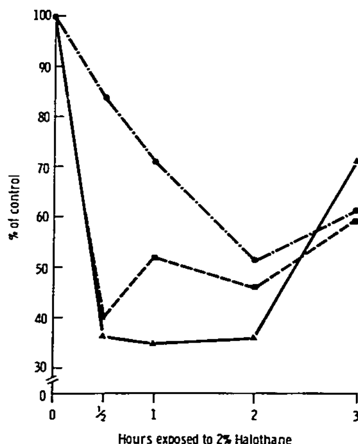

FIG. 9. The effects of 2 per cent halothane on the number of cells in different phases of the cell cycle. ▲——▲ = prophase; ●-·-·-● = metaphase; ■---■ = anaphase + telophase. Counts for three experiments were calculated as percentages of the corresponding control counts, then normalized to allow for different values for the mitotic index in parallel treated and control cultures, and finally combined as an average for each phase.

TABLE 4. Serial Changes in Mitotic Counts*

	Grand Mean of Controls	Exposure to 2 Per Cent Halothane						3 Hours of Exposure to Colchicine
		½ Hour	1 Hour	2 Hours	3 Hours	6 Hours	16 Hours	
Counts as percentage of all cells in mitosis								
Prophase	13	7	6	7	14	22	14	9
Metaphase	67	69	65	62	63	49	61	3
c-Metaphase	2	12	15	15	6	0	0	86
Anaphase + telophase	18	12	14	16	17	29	25	2
Mitotic index as percentage of all cells	4.9	2.9	3.0	2.6	3.3	2.3	1.5	11.9
Counts as percentage of all cells								
Prophase	0.64	0.20	0.18	0.18	0.46	0.51	0.21	1.07
Metaphase	3.28	2.00	1.95	1.61	2.08	1.13	0.92	0.36
c-Metaphase	0.10	0.35	0.45	0.39	0.20	0	0	10.23
Anaphase + telophase	0.88	0.35	0.42	0.42	0.56	0.67	0.38	0.24

* Values for ½–3 hours are the means of three experiments each; values for 6 and 16 hours represent single experiments.

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CFA are similar for halothane, but in the case of the other four agents ED_{50} for CFA is roughly twice the value for inhibition of cell division. This suggests that although the rate of cell increase was much reduced during the 24 hours of exposure to anesthetics, this was largely a temporary effect. When the anesthetic had been removed a dose-related proportion of the cells recovered and divided to form colonies that appeared to be normal.

A reduction in the rate of multiplication of cells could be due to a prolonged cycle time, to cell mortality, or to both. In the particular case of halothane, in our studies the concentration required for 50 per cent reduction of growth rate (2.19 per cent) was almost identical to the concentration that was found to double the measured cell cycle time (2.0 per cent) in the time-lapse film. It therefore seems likely that the effect on growth, after 24 hours of treatment at this concentration of halothane, is mainly due to a lengthening of cell cycle time.

In the present study we report a marked decrease in percentage of all cells in prophase within the first 30 minutes of anesthesia (fig. 9 and table 5). This can be explained only by a substantial decrease in the number of cells entering mitosis. The effect is most pronounced during the first 2 hours of anesthesia and, since this is less than the duration of G_2 (the interval between DNA synthesis and onset of mitosis: see fig. 1), it implies a delay in G_2, a conclusion which was also reached by Grant *et al.*,[23] working with root cells of *Vicia faba*.

Our data do not permit direct measurement of the duration of mitosis, but the period

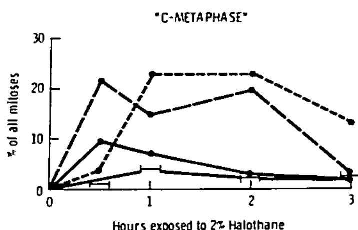

FIG. 10. Serial changes in c-metaphase counts expressed as percentages of all mitoses. The results of three experiments are shown and are contrasted with pooled control counts shown as horizontal bars.

between rounding-up and division is increased by 40 per cent, which implies a delay in mitosis. The pattern of mitosis was quite different from that produced by colchicine. With halothane the mitotic index fell, anaphase and telophase figures continued to appear in reduced numbers, and normal metaphases were still seen. This contrasts with the effect of colchicine, which increased the mitotic index and greatly reduced the number of normal metaphases, in place of which there was a very large number of c-mitotic figures. It appears that Chinese hamster fibroblasts respond differently from the root tip cells of *Vicia faba*, in which anesthetics caused an abnormality of mitosis similar to that produced by colchicine,[12] in line with the classic observations of Östergren,[11] who exposed various plants to large numbers of anesthetic agents and reported typical c-mitosis in all cases.

TABLE 5. Concentrations Needed for Anesthesia in Dogs (MAC), 50 Per Cent Inhibition of Cell Multiplication, and 50 Per Cent Inhibition of Colony-forming Ability (CFA)*

	MAC in Dogs†	ED_{50} for Cell Growth	ED_{50} for CFA‡	ED_{50} Growth / MAC	ED_{50} CFA / MAC
Methoxyflurane	0.23	0.65	1.1	2.8	4.8
Trichloroethylene	0.24§	1.01	2.7	4.2	11.3
Chloroform	0.77	1.06	2.1	1.4	2.7
Halothane	0.87	2.19	2.4	2.5	2.8
Diethyl ether	3.04	5.93	11.0	2.0	3.6

* All concentrations as percentages of one atmosphere.
† MAC in dogs taken from Eger *et al.*[27]
‡ Exposures to anesthetics lasted 24 hours.
§ MAC for trichloroethylene calculated from lipid-solubility data of Steward *et al.*[21]

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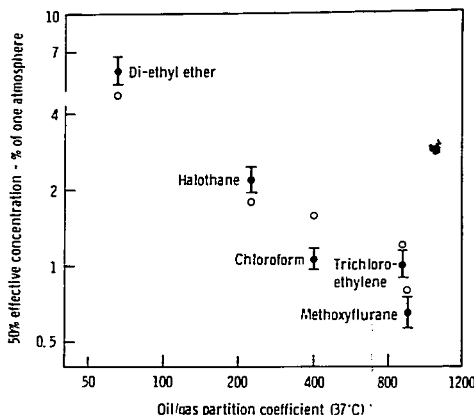

FIG. 11. Values for 50 per cent depression of cell multiplication (with 95 per cent fiducial limits) plotted against oil–gas partition coefficients for olive oil at 37 C. The hollow circles indicate the values obtained by Fink and Kenny.[16] Lipid solubility data are from Steward *et al.*[21]

The other main finding of this study was the not infrequent failure of division of the cytoplasm, leading to the formation of binucleate and trinucleate cells and a few multinucleate giant cells.

Elsewhere[24] we have reported that halothane causes a dose-dependent decrease in the rate of uptake of tritiated thymidine into DNA, and interference with DNA synthesis has been proposed by several other investigators.[14,15,25,26] We also presented evidence for prolongation of the G_1 phase (see fig. 1) caused by halothane in concentrations as low as 2 per cent (and as low as 1 per cent in later unpublished experiments). Grant *et al.*[23] did not exclude the possibility of an action in G_1.

Thus, it appears that anesthetics act on every phase of the cell cycle. It is not clear whether any common mechanism explains all the observed effects, and further research is required to elucidate the inhibition of adenosine triphosphate synthesis and calcium transport in relation to events in the cell cycle. Fink and Kenny[16] have shown a transition from aerobic to anaerobic metabolism in cell cultures exposed to halothane concentrations in the range 0.8–1.6 per cent. There is no evidence that action on one particular phase of the cell cycle is primarily responsible for the overall effect of anesthetics on cell multiplication. Therefore, it seems probable that in Chinese hamster fibroblasts the effects of anesthetics in lengthening the G_1 and G_2 phases cause the major reduction of cell multiplication rate during the time of treatment, and that abnormal mitoses result in dose-dependent cell killing, both during treatment and also later.

Finally, it should be stressed that we have adduced no evidence to suggest that any of the effects we observed would occur with the trace concentrations of anesthetics found as contaminants of operating theater atmospheres. The shape of the dose–response curves suggests that there would be negligible inhibition of growth by the concentrations detected under these conditions. However, exposure to trace concentrations in operating theaters takes place over prolonged periods, and there is the possibility of retention of long-lived products of biotransformation.

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