free neuromasts of teleosts. These are elongated flexible structures with definite form, similar to those described by others (*1*, *13*). It is likely that the cupula of the free neuromast in sharks, like that of the canal neuromast, is a mound of mucus which is continually being replenished. Probably it differs in texture and is more readily lost during handling or preservation (or both) than the typical tongue-like cupula of free neuromasts in teleosts.

ALBERT L. TESTER
JAMES I. KENDALL
Department of Zoology,
University of Hawaii, Honolulu

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Mitotic Synchrony in Mammalian Cells Treated with Nitrous Oxide at High Pressure

Abstract. *Mammalian cells grown in suspension or monolayer cultures were synchronized for cell division by the application of nitrous oxide under pressure. The metaphase block induced by nitrous oxide was dependent on pressure and was reversible. Exposure of HeLa cells to nitrous oxide had no significant effect on the synthesis of DNA, RNA, or protein. The progress of cells through the mitotic cycle was also unaffected. A high degree of mitotic synchrony was obtained in suspension cultures of HeLa cells treated with thymidine during exponential growth, resuspended in fresh medium, and then exposed to nitrous oxide.*

There are a number of procedures for obtaining synchronous (*1*) or synchronized (*2*, *3*) populations of mammalian cells in culture. I now describe a new method in which nitrous oxide (N_2O) applied at high pressures synchronizes cell division in mammalian cells grown as suspension or monolayer cultures.

Nitrous oxide at ordinary atmospheric pressures induced mitosis similar to that seen after treatment with colcemid (c-mitosis) in *Pisum sativum* but not in *Allium cepa* (*4*). The suggestion that similar effects might be produced in *Allium cepa* at higher pressures was proved to be correct by Ferguson *et al.* (*5*), who found that the threshold pressure for nitrous oxide to induce polyploidy was about 6 atm. I therefore tested N_2O for its usefulness in obtaining synchronous division in HeLa cells grown as suspension or monolayer cultures.

HeLa cells were maintained in exponential growth at 37°C in Eagle's minimal essential medium supplemented with nonessential amino acids, sodium pyruvate, and calf serum (5 percent). The cultures were gassed with a mixture of carbon dioxide (2.7 percent) and air. Monolayer cultures of HeLa (wild type) and a Fernandes line of human amnion cells were also maintained under similar conditions. The serum content of medium for human amnion cells was 10 percent, and 5 percent for others.

HeLa cells growing in spinner bottles or plastic tissue-culture dishes were exposed to N_2O in a stainless-steel pressure chamber (*6*). In experiments with former cultures, the cells were kept in suspension by a magnetic stirrer placed under the water bath directly beneath the pressure chamber. Optimum temperature was maintained by immersing the pressure chamber in a water bath kept at 38° ± 0.1°C for 1 day before and during the course of the experiment. One atmosphere of air, into which 20 ml (or 3 percent of the total volume) of carbon dioxide was injected, was allowed to remain in the pressure chamber before N_2O under pressure was introduced.

Cells fixed and stained as previously described (*3*) were examined microscopically. Nitrous oxide at high pressures blocked HeLa cells in exponential growth in metaphase. On microscopic examination these blocked cells presented typical c-mitotic configurations. The effectiveness of the N_2O block was dependent on the pressure. The metaphase block was incomplete between 2.72 and 4.42 atm and was ineffective at lower pressures. In the range of 2.72 to 4.42 atm, most of the cells could complete mitosis, which was anomalous in some respects and appeared to be similar to the phenomenon of chromatid nondisjunction (*7*). The optimum range of pressure for a complete metaphase block was 5.1 to 5.4 atm of N_2O. Increasing the pressure above 6.8 atm or the duration of treatment beyond 16 hours, even in the range of 5.1 to 5.4 atm, caused disintegration of some cells. Nitrogen applied at similar pressures caused no metaphase block.

The metaphase block was reversible. The reversibility was greatly influenced by the duration of exposure and pressure. A partially synchronized population of HeLa cells was obtained by treating an exponential culture with thymidine (final concentration, 2.5 mmole/liter) for 16 hours, centrifuging

Table 1. Effect of N_2O on the progression of cells through mitotic cycle; M.I., mitotic index; labeled, percentage of cells labeled with tritiated thymidine.

Duration of treatment (hours)	Nitrous oxide at 5.1 atm		Control with colcemid		Control without colcemid	
	M.I.	Labeled (%)	M.I.	Labeled (%)	M.I.	Labeled (%)
1.0	0.03	35.0	0.03	39.0	0.02	36.0
2.0	.08	51.0	.06	47.5	.04	49.5
4.0	.11	55.0	.12	55.0	.03	58.0
8.0	.27	78.0	.26	78.0	.03	79.5
16.0	.62	80.0	.65	81.5	.03	83.0

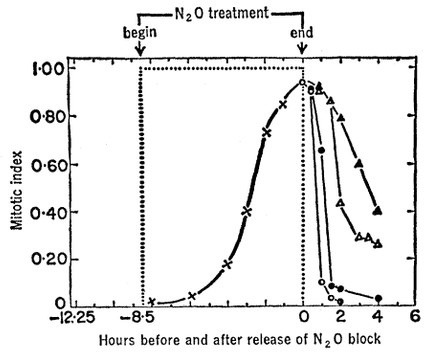

Fig. 1. Effect of nitrous oxide on mitosis and its reversal. An exponential culture of HeLa cells treated with excess thymidine for 16 hours was resuspended in fresh medium at −12.25 hours; 3.75 hours later they were exposed to N_2O (5.1 atm) for 8.5 hours as indicated by the dotted enclosure. Accumulation of mitoses during N_2O treatment, ×. The rate of decrease in mitotic index was studied as a measure of reversibility in cultures exposed to N_2O (5.1 atm) for various periods commencing 3.75 hours after the reversal of the thymidine block. The duration of N_2O treatments were: 8.5 hours, ○; 12 hours, ●; 16 hours, △; and 20 hours, ▲.

the cell suspension, and resuspending the cells in regular medium. At 3.75 hours after the reversal of the thymidine block, when the wave of mitotic activity was about to commence, the spinner bottle with 100 ml of cell suspension was placed in the stainless-steel chamber and N_2O (5.1 atm) was applied. Within 8.5 hours the mitotic index rose from 0 to about 0.94 (Fig. 1). One-fourth of the cell suspension (25 ml) was taken out after 8.5, 12, 16, and 20 hours of exposure and kept in an incubator. In these samples, the mitotic index was measured at 30-minute intervals and plotted (Fig. 1). The data indicate that the longer the exposure to N_2O, the slower the rate of reversal. The rate of reversal of the mitotic block was measured as the decrease in the mitotic index (or the ability of cells to complete mitosis and enter interphase) as a function of time after the removal of the culture from the N_2O chamber. In cells exposed to N_2O at 7.48 atm or more, the reversal of the block was slower, and in some cells the mitoses were aberrant; a few chromosomes were not included in the metaphase plate.

Such a high degree of mitotic accumulation and a highly synchronous cell division which resulted in a precipitous drop in mitotic index indicated that large quantities of synchronized cells could be obtained. An exponential culture was treated with an excess of thymidine (2.5 mmole/liter) for 16 hours; 3.75 hours later they were exposed to N_2O (5.1 atm). Cells thus synchronized were studied with regard to DNA synthesis period (S-period) and the degree of synchrony during the second burst of cell division. After the reversal of the N_2O block, cell samples were taken at regular intervals and exposed to ^{3}H-thymidine (0.1 μc/ml; 2.0 c/mmole) for 20 minutes. The cell samples were fixed and processed for radioautography (*8*). The advancement of cells toward the second wave of mitosis was measured as the rate of mitotic accumulation in the presence of colcemid. Colcemid (6.7×10^{-7} mole/liter) was added after 10 hours. The cells were in excellent synchrony (Fig. 2); about 90 percent of cells completed mitosis within 1 hour after the release of the N_2O block. The synchronous cell division was followed by a fairly synchronized period of DNA synthesis, indicated by a sharp increase in the percentage of cells labeled with ^{3}H-thymidine. After 13 hours, about 90 percent of the cell population was in the S-period. However, due to cellular variability, the degree of synchrony decayed somewhat by the time the cells reached the second wave of mitosis (Fig. 2).

Tritiated thymidine (0.05 μc/ml; 2.0 c/mmole) was added to an exponential culture of HeLa cells, and 5 ml (about 10^6 cells) of cell suspension was dispensed into each of 15 plastic culture dishes. The dishes were grouped into sets of five each. One set was transferred to the pressure chamber with N_2O (5.1 atm). The second, to which colcemid (6.7×10^{-7} mole/liter) was added to stop further increase in cell number, and the third (without colcemid) were kept in the incubator at 37°C. One dish from each of the three sets was taken out after 1, 2, 4, 8, and 16 hours; and the cells were detached by treatment with trypsin, fixed, and processed for radioautography. The mitotic index and the percentage of labeled cells after exposure to N_2O at 5.1 atm for various periods of time were similar to those in controls treated with colcemid (Table 1). In the third group the percentage of labeled cells was identical with that of the other sets, but the mitotic index remained constant at about 0.03. The treatment of HeLa cells with N_2O at 5.1 atm had no effect on the rate of flow of cells from the period before DNA synthesis (G1) into S-period or on the entry of interphase

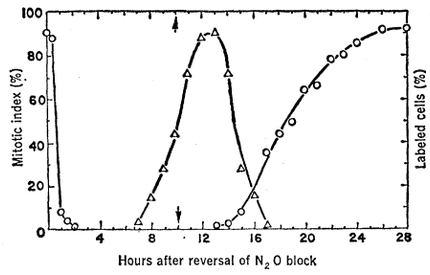

Fig. 2. The kinetics of cell division and DNA synthesis after the reversal of nitrous oxide block. The second wave of cell division was blocked by the addition of colcemid (6.7×10^{-7} mole/liter) at 10 hours (arrow). Mitotic index (percent), ○; percentage of cells labeled during an exposure to ^{3}H-thymidine for 20 minutes, △.

cells into mitosis (Table 1). The uptake of tritiated thymidine, uridine, or methionine into cellular DNA, RNA, or protein as determined by scintillation counting was not significantly different in cells exposed to N_2O at 5.1 atm for 16 hours and controls treated with colcemid.

The yield of mitotic fraction from monolayer cultures by selective detachment could be improved significantly after an 8-hour exposure to N_2O at 5.1 atm. During the 8-hour treatment of HeLa (wild type) or human amnion cell lines which have a generation time of about 22 hours, nearly 30 percent of the cells would be trapped in metaphase. This fraction would be even greater in cell lines with shorter generation times. In contrast, the treatment with biochemical mitotic inhibitors should be limited to relatively shorter periods [about 2 to 4 hours in the case of colcemid (*9*)], as exposures to these drugs beyond this time would cause aberrant mitosis or irreversible mitotic block.

POTU N. RAO

Department of Physiology and Biophysics, University of Kentucky Medical Center, Lexington

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Exsolution Lamellae and Optic Orientation of Clinoamphiboles

Abstract. *Exsolution lamellae are abundant in coexisting hornblende and cummingtonite, and in hornblende coexisting with anthophyllite in Ordovician volcanics metamorphosed in the kyanite and sillimanite zones in central Massachusetts and adjacent New Hampshire. The lamellae have the same orientation relative to the internal structure as the (100) and (001) exsolution lamellae in clinopyroxenes, but are indexed (100) and ($\bar{1}01$) with the* C2/m *space group commonly chosen for amphiboles. Specimens from the kyanite zone contain very thin (100) and ($\bar{1}01$) lamellae. In the sillimanite zone, both (100) and ($\bar{1}01$) lamellae are thicker and more abundant in iron-rich specimens than they are in magnesian specimens, as might be expected by analogy with pyroxenes from layered mafic intrusions. The ($\bar{1}01$ lamellae allow correct determination of the relations between the optic vibration directions and the crystallographic axes for two alternatively selected space group* C2/m and I2/m. *This evidence shows that there has been much confusion concerning these relations.*

Thin (001) lamellae of hornblende in cummingtonite and cummingtonite in coexisting hornblende have been observed in amphibolites from Queensland, Australia (*1*), Sweden (*2*), and the Orange area of Massachusetts and New Hampshire (*3*). We have found thin lamellae parallel to (100) in amphiboles from the Orange area. The lamellae parallel to the (100) planes of cummingtonite are green hornblende, and those in hornblende are cummingtonite, at least where thick enough for extinction to be observed. Hornblendes from New South Wales (*4*) contain (001) exsolution plates of cummingtonite and also very thin (100) plates that were considered to be anthophyllite by analogy to pyroxene exsolution phenomena. Amphibolites from Italy (*5*) contain lamellae identical to those we describe here. However, single crystal studies (*6*) on one of our specimens [(I38A), not described here] and additional work on another [(7A8B), described in detail here] show that the two sets of lamellae in hornblende and cummingtonite should be indexed (100) and ($\bar{1}01$) when *C2/m* is chosen as the space group.

We now report amphiboles occurring in the Orange area (*3*, *7*) in metamorphosed volcanics of the Ammonoosuc and Partridge formations of Middle Ordovician age that are involved in nappes and gneiss domes of a portion of the Bronson Hill Anticlinorium. Electron-microprobe analyses of 16 pairs of coexisting amphiboles have been made (*8*). Many of the analyzed clinoamphiboles contain abundant exsolution lamellae. Coexisting cummingtonite and hornblende from the kyanite zone [(100 × Fe)/(Fe + Mg) = 40 to 55] contain very thin (0.2 to 0.3 μ) lamellae of each other parallel to (100) and ($\bar{1}01$) of the host. In the sillimanite zone, magnesian hornblendes [(100 × Fe)/(Fe + Mg) = 24 to 30] associated with anthophyllite contain very thin (0.2 to 0.4 μ) cummingtonite lamellae parallel to (100) and thin lamellae (0.3 to 0.5 μ) parallel to ($\bar{1}01$). Both sets of lamellae tend to be coarser (0.4 μ and 0.8 to 1.0 μ) and more abundant, with higher iron content in the same assemblage [(100 × Fe)/(Fe + Mg) = 38 to 40]. Coexisting cummingtonites and hornblendes [(100 × Fe)/(Fe + Mg) = 40 to 55] contain still coarser lamellae (0.5 to 0.6 μ and 1.5 to 2.0 μ) parallel to (100) and ($\bar{1}01$) and have proved most suitable for optical study. Increasing

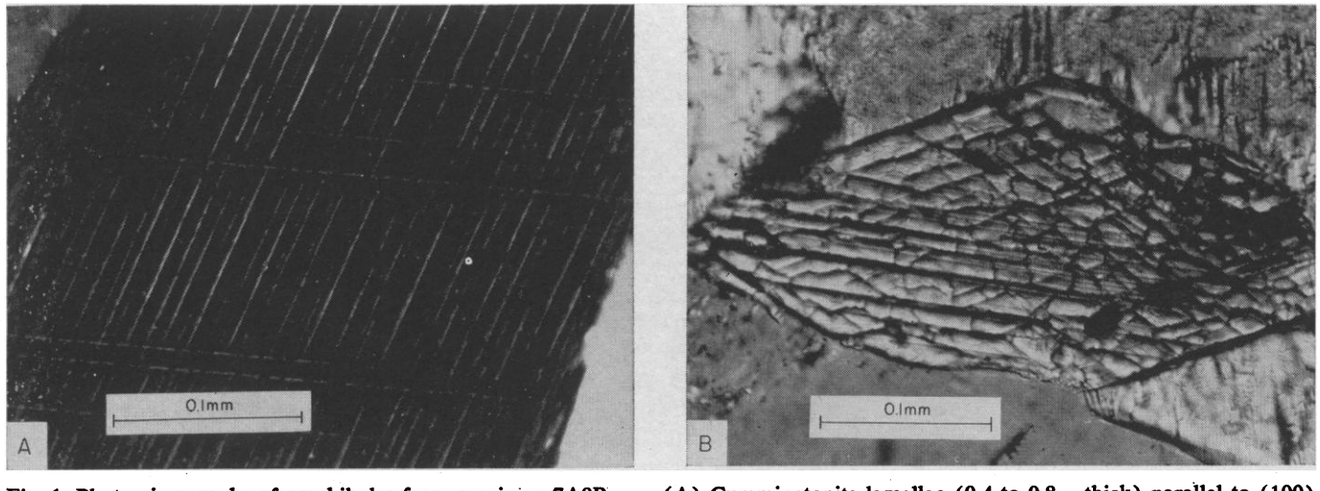

Fig. 1. Photomicrographs of amphiboles from specimen 7A8B. (A) Cummingtonite lamellae (0.4 to 0.8 μ thick) parallel to (100) and to ($\bar{1}01$) of host hornblende at extinction under crossed nicols. The lamellae are illuminated because the *c* axes and *Z*-vibration directions of the two amphiboles are not parallel. Lamellae parallel to (100) are thinner (0.4 μ) than ($\bar{1}01$) lamellae, taper close to intersections with ($\bar{1}01$) lamellae, and are offset by the ($\bar{1}01$) lamellae. (B) Cummingtonite section cut parallel to ($\bar{1}01$) plane. Thin (0.5 μ) lamellae of hornblende exsolved parallel to the (100) plane of cummingtonite appear as striations bisecting the {110} cleavages. Plane polarized light.