(Academic Press, New York, 1966), p. 332.
4. G. Ostergren, *Hereditas* **30**, 429 (1944).
5. J. Ferguson, S. W. Hawkins, D. Doxey, *Nature* **165**, 1021 (1950).
6. Purchased from Parr Instruments Company, Moline, Illinois.
7. P. N. Rao and J. Engelberg, *Exp. Cell Res.* **43**, 332 (1966).
8. ———, *Science* **148**, 1092 (1965).
9. E. Stubblefield and R. Klevecz, *Exp. Cell Res.* **40**, 660 (1965); P. N. Rao and J. Engelberg, *ibid.* **48**, 71 (1967).

10. I thank Dr. R. Hinegardner for the suggestion that nitrous oxide might be useful as a reversible mitotic inhibitor and Dr. J. Engelberg for the help in setting up the pressure chamber and most helpful discussions during the course of this study. I thank Dr. J. Sisken for supplying the starter culture of a Fernandes line of human amnion cells and Mrs. N. Mitchell and Mrs. M. Mitchell for their technical assistance. Supported by PHS grant NIH-CA-06835.

1 April 1968

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

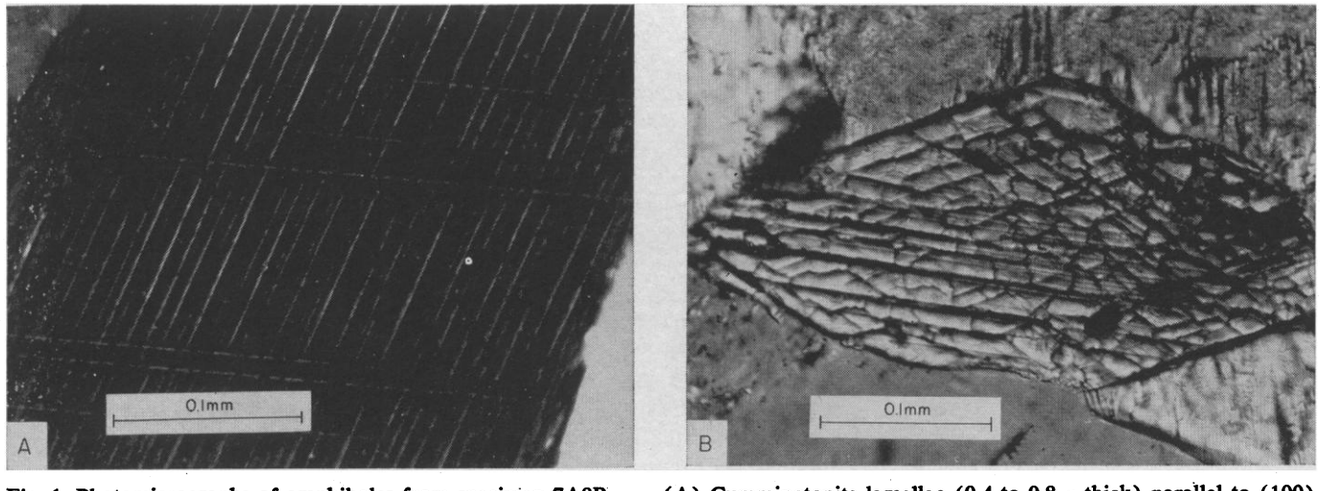

Fig. 1. Photomicrographs of amphiboles from specimen 7A8B. (A) Cummingtonite lamellae (0.4 to 0.8 μ thick) parallel to (100) and to ($\bar{1}01$) of host hornblende at extinction under crossed nicols. The lamellae are illuminated because the *c* axes and *Z*-vibration directions of the two amphiboles 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.

mutual solubility with increasing iron content before exsolution is expected by analogy to the inferred form of the pyroxene solvus (*9*). Coarse hornblende lamellae (0.5 μ and 1.3 μ) were found in retrograde cummingtonite [(100 × Fe)/(Fe + Mg) = 48] from the sillimanite zone in contact with secondary hornblende and magnesian chlorite.

The prominent ($\bar{1}01$) lamellae allow a correct determination of the relations between optic vibration directions and crystallographic axes. For this purpose, specimen 7A8B (Fig. 1) was most suitable. Partial electron-microprobe analyses of coexisting cummingtonite and hornblende hosts from sample 7A8B gave, respectively, SiO_2 52.3, 44.1; Al_2O_3 2.1, 12.0; total Fe as FeO 25.1, 20.2; MnO 0.6, 0.3; MgO 14.9, 9.3; CaO 1.4, 9.0; Na_2O 0.2, 1.9; total, 96.6, 96.8; (100) × Fe/(Fe + Mg) 48.6, 54.9. Single-crystal measurements on a grain consisting of 75 percent cummingtonite and 25 percent hornblende gave respective values of *a* 9.51 Å, 9.82 Å; *b* 18.15 Å, 18.15 Å; *c* 5.31 Å, 5.33 Å; *a* sin β 9.30 Å, 9.49 Å; c ∧ ($\bar{1}01$) lamellae 109.6°, 106.5°. Most of the hornblende occurs as a concentration on one end of the grain joined to the cummingtonite along a common ($\bar{1}01$) plane and as ($\bar{1}01$) exsolution lamellae in the cummingtonite. Weaker reflections from hornblende lamellae oriented parallel to (100) of cummingtonite gave similar cell dimensions. Optical properties of the cummingtonite and hornblende hosts are, respectively: γ 1.675 ± 0.001, 1.692 ± 0.002; β 1.662 ± 0.001, 1.684 ± 0.002; α 1.648 ± 0.001, 1.699 ± 0.001; $\gamma - \alpha$ 0.028, 0.023; $Z \wedge c$ 20° ± 1°, 16° ± 1°; $Z \wedge (\bar{1}01)$ lamellae 92° ± 1°; 94° ± 1°, $c \wedge (\bar{1}01)$ lamellae 112° ± 1°, 110° ± 1°; $2V$ (calc.) 89°+, 72°−; and dispersion $r < v$ weak, $r > v$ weak.

The optic orientations are illustrated in Fig. 2. Where individual crystals of hornblende and cummingtonite are intergrown along a common ($\bar{1}01$) plane their prismatic cleavages and (100) exsolution lamellae are not parallel but meet at an angle of 178° ± 1°, as expected from their different β angles. This difference of 2° in orientation nearly compensates for their different $Z \wedge c$ angles, so that their *Z*-vibration directions are nearly parallel. There is a discrepancy of 2° or 3° in the angle $c \wedge (\bar{1}01)$ between x-ray single-crystal measurements by Ross and our microscopic measurements. Repeated microscopic measurements by various means have consistently given values of this angle close to 112° for cummingtonite and 110° for hornblende. We believe this discrepancy is real, but cannot account for it. The lamellae may represent the angle $c \wedge (\bar{1}01)$ under the temperature-pressure conditions at which exsolution took place; this angle may have since decreased in the crystal lattice to the values obtained in the x-ray measurements, either by gradual shifting, or by an abrupt inversion. The latter would be indirectly analogous to orthopyroxenes in layered mafic intrusions that are believed to be inverted pigeonite and contain exsolution lamellae oriented appropriately for (001) of pigeonite (*9*).

If the exsolution lamellae are assigned to ($\bar{1}01$), assuming space group $C2/m$, then one must conclude from Fig. 2 that the *Z*-vibration direction lies in the acute angle between the *a* and *c* crystallographic axes, rather than in the obtuse angle as shown in all standard references (*10*), a brief survey of which shows a variety of assumed relations mainly inconsistent with our data.

The relations between optical vibration directions and crystallographic axes for amphiboles used in most optical references are based on studies of terminated crystals in which the angle β between the *c* axis and a well developed (001) terminal face can be measured. This β is the same as that assigned for the *I* cell by x-ray measurements. In x-ray studies, where identification of lattice type is possible, it is traditional to reduce all nonprimitive monoclinic lattices to the *C* lattice and to give the β angle appropriate to this lattice. In most amphiboles, the cell with the three shortest edges is the *C* cell, but in most pyroxenes it is the *I* cell. The confusion in the standard references (*10*) results directly from failures to make the necessary connections between

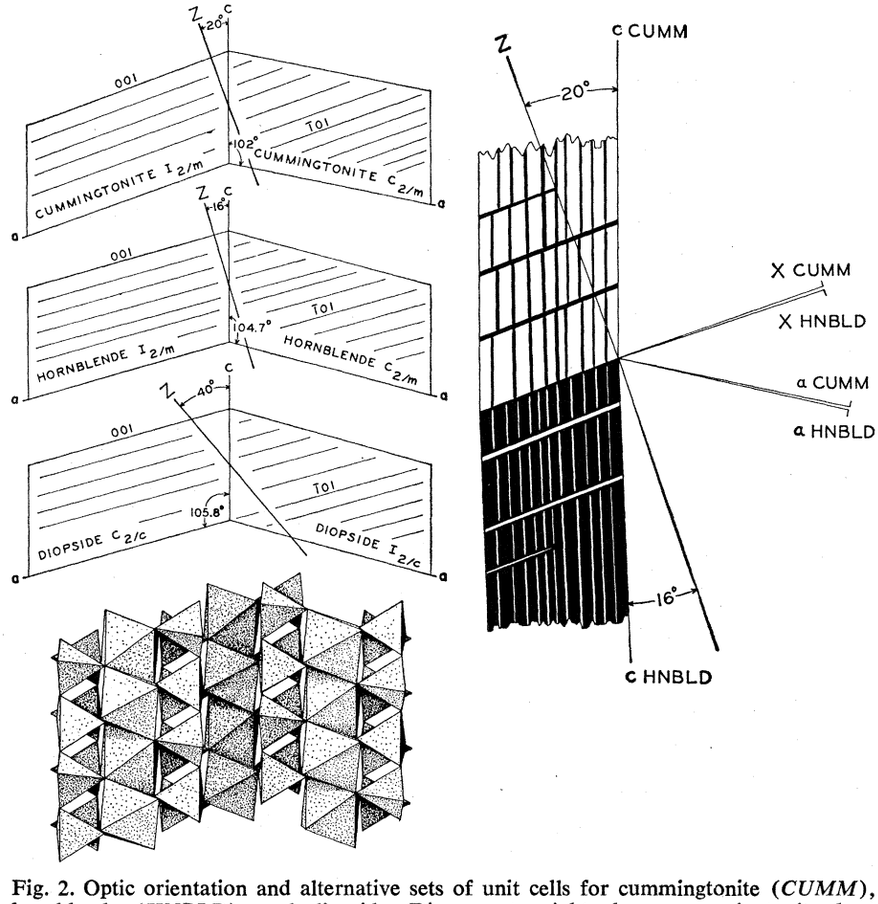

Fig. 2. Optic orientation and alternative sets of unit cells for cummingtonite (*CUMM*), hornblende (*HNBLD*), and diopside. Diagram to right shows cummingtonite host (white) and hornblende host (black) in contact along ($\bar{1}01$) plane with both phases showing exsolution lamellae. Exsolution lamellae parallel to (100) and traces of {110} cleavages (not shown) meet at 178° angle at contact between hosts. Alternate cells with *Z*-vibration directions and exsolution planes are shown to left, and are compared with an oriented portion of the internal structure that is similar in all three minerals. The drawing is adapted from the structure of diopside (*11*) and the octahedra shown are M_1. For amphiboles the corresponding octahedra are M_1 and M_2.

morphological, optical, and x-ray crystallography.

Study of structure determinations of diopside (*11*) and tremolite (*12*) and packing models of both shows that the amphibole space group directly comparable to diopside $C2/c$ is $I2/m$. Lamellae directly comparable to (001) in pyroxene would be indexed as $(\bar{1}01)$ for space group $C2/m$ of amphibole. Similarly, if the C cell is chosen for the amphibole, then the directly corresponding pyroxene space group is $I2/c$. Figure 2 shows the relations between these two alternative sets of pyroxene and amphibole cells together with the Z-vibration directions and exsolution planes. The traditional optic orientation of diopside was independently confirmed on a chromian specimen from Outokumpu, Finland, showing excellent {001} parting. The angle $Z \wedge c$ has the same sense relative to the internal structure in both diopside and clinoamphibole.

HOWARD W. JAFFE
PETER ROBINSON
Department of Geology, University of Massachusetts, Amherst 01002
CORNELIS KLEIN, JR.
Department of Geological Sciences, Harvard University, Cambridge, Massachusetts 02138

References and Notes

1. R. H. Vernon, *Amer. Mineral.* **47**, 360 (1962).
2. B. Asklund, W. L. Brown, J. V. Smith, *ibid.*, p. 160.
3. P. Robinson, thesis, Harvard University (1963).
4. R. A. Binns, *Mineral. Mag.* **35**, 306 (1965).
5. E. Callegari, *Estratto dalle Memorie della Accademia Patavina di Scienze, Lettere ed Arti, Classe di Scienze Matematiche e Naturali* **78**, 273 (1965–66).
6. M. Ross, J. J. Papike, P. W. Weiblen, *Geol. Soc. Amer. Ann. Mtg.* 1967, abstr., p. 190; *Science* **159**, 1099 (1968).
7. P. Robinson, *Guidebook New England Intercollegiate Geological Conference, Amherst, Mass.* (1967), p. 17.
8. C. Klein, *Geol. Soc. Amer. Ann. Mtg.* 1966, abstr., p. 110; *J. Petrol.*, in press.
9. A. Poldervaart and H. H. Hess, *J. Geol.* **59**, 472 (1951).
10. W. A. Deer, R. A. Howie, J. Zussman, *Rock-Forming Minerals* (Wiley, New York, 1963), vol. 2, p. 234; A. N. Winchell and H. W. Winchell, *Elements of Optical Mineralogy* (Wiley, New York, ed. 4, 1951), vol. 2, p. 427; W. E. Tröger, *Optische Bestimmung der gesteinbildenden Minerale* (Schweizerbart, Stuttgart, 1959), vol. 1, p. 65; L. G. Berry and B. Mason, *Mineralogy* (Freeman, San Francisco, 1959), p. 523; E. W. Heinrich, *Microscopic Identification of Minerals* (McGraw-Hill, New York, 1965), p. 249.
11. B. E. Warren and W. L. Bragg, *Z. Krist.* **69**, 168 (1928); N. Morimoto, D. E. Appleman, H. T. Evans, Jr., *ibid.* **114**, 120 (1960).
12. B. E. Warren, *ibid.* **72**, 42 (1929); J. Zussman, *Acta Cryst.* **12**, 309 (1959).
13. Supported by a University of Massachusetts faculty research grant and by NSF research grants GA-467 and GA-1085 (the latter to J. B. Thompson, Jr.). We thank M. Ross for discussions and preliminary information concerning his x-ray studies of amphiboles.

8 March 1968

Sterol Requirement for Reproduction of a Free-Living Nematode

Abstract. *The free-living, hermaphroditic nematode* Caenorhabditis briggsae *has a nutritional requirement for sterols. It will reproduce indefinitely in a liquid medium containing only bacterial cells* (Escherichia coli) *and salts if various sterols are present. Several other lipid-soluble materials are ineffective in supporting reproduction.*

Reproduction of free-living nematodes under axenic conditions requires the presence of an undefined tissue extract (*1*). Mammalian liver or chick embryo are the most appropriate sources (*2*), though extracts of other tissues and bacteria are reportedly effective (*3*). Although a suitable defined basal medium for the organisms has been developed, the nutritional nature of the extract has not been satisfactorily resolved.

Fractionation of an extract of lamb liver on hydroxylapatite has led to the recovery of fractions which support growth and reproduction of free-living nematodes (*3*). These fractions consist mostly of protein, but they also contain a small lipid moiety. Since the basal medium contains no lipids (*4*), the nematodes can either synthesize all of their required sterols and fatty acids or these materials must be provided by the "growth factor." The free-living nematode *Turbatrix aceti* can synthesize polyunsaturated fatty acids *de novo* (*5*), but no evidence was found for sterol biosynthesis in this organism or in *C. briggsae* (*6*).

Caenorhabditis briggsae, a hermaphroditic nematode, grows and reproduces in the presence of *Escherichia coli* (and other bacteria) on a nutrient agar medium. However, Dougherty (*7*) reported that the nematode will not reproduce if the nutrient agar is changed to one containing only basal salts and glucose. These results suggest that essential metabolites lacking in the bacteria are provided in the nutrient agar. Particularly significant is the fact that procaryotic bacteria are deficient in sterols and polyunsaturated fatty acids (*8*).

Axenic larvae of *C. briggsae* were inoculated into sterile, capped test tubes (13 by 100 mm) containing slants of minimal agar previously streaked with *E. coli* (ML30 strain) grown on a minimal medium consisting of salts and glucose. A purified grade of agar (Difco Purified Agar) was used and contained the following: 8.5 g of K_2HPO_4, 4.2 g of KH_2PO_4, 1.0 g of NaCl, 0.8 g of NH_4Cl, 0.1 g of Na_2SO_4, 0.05 g of $MgCl_2 \cdot 6H_2O$, 0.01 g of $CaCl_2$, 0.0005 g of $FeSO_4 \cdot 7H_2O$, 1.0 g of glucose, and 16 g of agar per liter of distilled water, adjusted to *p*H 6.9 to 7.0. The nematodes grew and reproduced in 5 days at 20°C. The offspring were serially subcultured four times without diminishment of growth. Thus, it appeared that our agar possessed an essential nutrient lacking in Dougherty's original preparation (*7*), or that the nematodes could grow successfully on *E. coli* as the sole source of organic nutrients. However, larvae of *C. briggsae*, when inoculated into a liquid medium limited to phosphate buffer and *E. coli* (see below) reproduced readily for only one generation. The offspring usually grow only to advanced larval stages or to the size of small adults. An occasional individual (about one out of six) managed to produce new larvae which did not survive further subculture in this medium.

To show that the material lacking in the liquid medium is a sterol, we carried out the following experiments. *Escherichia coli* (ML30) was grown in a minimum liquid medium with the same composition as that of the agar slants (except that 2.5 g of glucose per liter was added) for 9 hours at 37°C. The cells were collected, washed by centrifugation, and finally diluted with a .067*M* potassium phosphate buffer–salt solution containing in final concentration: 3.25 m*M* NaCl, 3.0 m*M* magnesium citrate, 1.5 m*M* $CaCl_2$, 0.235 m*M* Na_2SO_4, 0.15 m*M* $FeCl_3$, 0.112 m*M* $MnCl_2$, 0.075 m*M* $ZnCl_2$, and 0.038 m*M* $CuCl_2$. The concentration of bacterial cells was approximately 0.3 mg/ml (dry weight).

Stoppered tubes (10 by 75 mm) containing 0.25 ml of cell suspension were each inoculated with three freshly hatched larvae and incubated at 20°C. The offspring from these cultures were subcultured into fresh medium of the same composition. From the occasional individual which again reproduced, "depleted" larvae were obtained for the growth experiments outlined in Fig. 1. Cholesterol, 7-dehydrocholesterol, ergosterol, β-sitosterol, and stigmasterol were combined and dissolved in Tween 80, and the product was sterilized by Millipore filtration. The