Table 2. Distribution of Fe^{2+} in clinoamphiboles.

Specimen	Fe^{2+} (%)	Total Fe (per formula unit) (%)	Position of Fe			
			In M_1, M_3	Per M_1, M_3	In M_2 or M_2, M_4	Per M_2 or M_2, M_4
Tremolite-ferroactinolite series						
Berkeley 14785	15.2	0.76	0.32	0.11	0.44	0.22
USNM 80714	30.0	1.50	.62	.21	.88	.44
Mueller, 12BA	47.9	2.40	1.56	.52	.84	.42
Cummingtonite-grunerite series						
USNM 118125	36.0	2.52	0.72	0.24	1.80	0.45
Mason	53.4	3.74	1.50	.50	2.24	.56
Klein, 7	65.1	4.56	1.96	.65	2.60	.65
Klein, 1	88.8	6.22	2.56	.85	3.66	.92
Mueller, 1B	98.0	6.86	2.95	.98	3.91	.98

polarization-dependence of the hydroxyl peak in phlogopite (*6*) is similar to that in the amphiboles.

The absence of overtone contributions in the polarized β spectra indicates that the O-H bond lies in the (010) plane. Its direction in this plane may be calculated from the observed ratio of absorbances for light vibrating $\|\alpha$ and $\|\gamma$ by applying the relation

$$\sin^2\theta/\cos^2\theta = A_\alpha/A_\gamma$$

where θ is the angle between α and the O-H bond. From the ratio $A_\alpha/A_\gamma = 22/5$, the value of θ is 24° ± 6°. The angle γ/z at the overtone frequency is 19° ± 2° in the obtuse angle beta (β = 105°), so that the O-H bond makes an angle of [90 − (24±6 − 19±2)] = 85° ± 8°, with z in the (010) plane. Inspection of the structure model suggests that the bond is, in fact, normal to z. If this is so, sections of tremolite (which is obtainable in large single crystals) normal to z would be very efficient polarizers over narrow bands centered on 3673 and 7180 cm^{-1}.

The relative intensities of peaks *A*, *B*, *C*, and *D* may be used to estimate the distribution of Fe^{2+} between the M_2 and (M_1,M_3) positions in the tremolite-ferroactinolite series, and between the (M_2,M_4) and (M_1,M_3) positions in the cummingtonite-grunerite series, provided the total iron content is known.

$$\Sigma Fe^{2+}\ (M_1M_3) = 0{\cdot}A + 1{\cdot}B + 2{\cdot}C + 3{\cdot}D$$

$$\Sigma Mg^{2+}\ (M_1M_3) = 3{\cdot}A + 2{\cdot}B + 1{\cdot}C + 0{\cdot}D$$

The proportion of Fe^{2+} ions in (M_1M_3) positions is $3\Sigma Fe/(\Sigma Fe + Mg)$, and the number in M_2 or (M_2 + M_4) positions is obtained by difference (Table 2). The results indicate that the Fe/(Fe + Mg) ratio in the M_2 position is approximately twice that in the (M_1,M_3) positions in actinolites with up to 30 percent F^{2+}, and that the (M_2,M_4) positions are similarly favored in the cummingtonite-grunerite series. Similar conclusions were reached by less direct x-ray methods (*4*, *7*).

The spectrum of a manganiferous cummingtonite (*8*) showed no evidence of the presence of Mn^{2+} in the (M_1, M_3) positions, but all peaks were shifted to higher frequencies by 2 to 5 cm^{-1}, suggesting that the large Mn^{2+} ion, like Ca^{2+}, enters the M_4 position. In chemically more complex amphiboles, increasing numbers of peaks of ever-decreasing intensity appear, and in common hornblende the O-H fundamental is a single, broad, jagged band.

Determination of the orientation of the O-H bond from overtone bands (when present) rather than the fundamental has the advantage that conventional petrographical thin sections can be used, enabling minerals with good cleavage to be studied in any orientation. Previous single-crystal work with the fundamental band has been largely confined to minerals of which suitable cleavage flakes could be obtained, and orientations were restricted to zero or low-tilt angles (*6*). The microscope technique should prove applicable to a wider variety of minerals, although limited to those with overtone bands.

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2 May 1966

Selection of Sucrose-Dependent Escherichia coli To Obtain Envelope Mutants and Fragile Cultures

Abstract. *Mutants isolated as sucrose-dependent include many with apparent envelope defects, which fact often leads to filamentous growth or lysis in the absence of sucrose. One mutant can grow exponentially in 4 percent sucrose, but is very fragile: it releases all of its RNA and soluble protein when treated with 0.5 percent sodium deoxycholate. These characteristics permit the study of unstable structures and rapid processes in actively growing cells.*

Since the basis of bacterial rigidity is the cell wall and since 10 to 20 percent sucrose can stabilize otherwise unstable spheroplasts (*1*), it seemed probable that there is a class of mutants, with defects of the cell wall, which is dependent on high concentrations of sucrose for growth, and that some of these mutants would be fragile in its absence. We isolated such mutants.

Escherichia coli, strains K12 3000 B_1^- and AB 301 *met*$^-$, or a derivative of AB 301 constitutive for alkaline phosphatase, were grown exponentially at 37°C in trypticase soy broth to 2 × 10^8 cells per milliliter. Cultures were then treated with 30 μg/ml of the mutagens ethylmethane sulfonate or *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine for 30 minutes, and samples were diluted and plated on nutrient agar containing 20 percent sucrose. After replica plating on media with and without sucrose, colonies that grew only in the presence of sucrose were purified and tested in liquid medium.

Of 30 independent *sud* (sucrose-dependent) mutants that showed an absolute requirement for sucrose on plates, 22 required sucrose for growth

in liquid medium. Eight mutants grew equally well, for unknown reasons, in liquid medium in either the presence or absence of sucrose. Mutants that require sucrose in liquid culture have been maintained by monthly transfers on agar slants containing 20 percent sucrose.

The expectation that many of the mutants could exhibit defects in wall metabolism has been confirmed. For example, when cells from seven strains were centrifuged out of cultures growing in medium containing sucrose and resuspended in growth medium without sucrose, cells of one strain (*sud* 24) lysed after several minutes, while those of four others (*sud* 19, 21, 22, and 25) formed long filaments. Mutant *sud* 22, constitutive for alkaline phosphatase, was found to release 60 percent of the enzyme [which is ordinarily bound to the cell surface (*2*)] into the culture medium during exponential growth in medium containing 20 percent sucrose.

Sud 24 and *sud* 25 have been further examined. *Sud* 25 grows in minimal salts–glucose medium or in nutrient broth if they are fortified with either 20 percent sucrose or bacterial hydrolyzate (*3*). Subsequently it was found that, for *sud* 25, sucrose can be replaced by the bacterial wall component D-alanine.

Sud 24 satisfies the requirement for a strain that can be grown in fragile form. In media containing 11 to 20 percent sucrose, the strain grows at the same rate and is as difficult to lyse as the parental strain in all stages of growth. However, transferred into lower concentrations (3 to 5 percent), cells continue to grow but rapidly become fragile and begin to lyse. Figure 1 shows a family of growth curves for cells centrifuged out of 20 percent sucrose and resuspended in medium containing various lower concentrations. The lysis apparent in low concentrations of sucrose can be prevented by addition of 15 percent carbowax 4000 (*4*); carbowax itself prevents lysis but cannot promote growth in the absence of sucrose. The growth curve in 4 percent sucrose and 15 percent carbowax is exponential (inset of Fig. 1), and microscopic observations and colony counts show that cells in the culture have normal morphology and that they increase in numbers exponentially. Under these conditions, however, when cells are treated with the lysing agent sodium deoxycholate, they prove to be totally fragile: all the preformed β-galactosidase and RNA are released, and the light scattering of the culture disappears almost completely (Fig. 2).

Table 1 shows a comparison of the relative efficiencies of various lysing media for each of three parameters of lysis. We have noted repeatedly that the release of RNA is the most sensitive

Table 1. Relative efficiency of lysing agents. *Sud* 24 was grown in medium containing 4 percent sucrose and 15 percent carbowax 4000 (see inset of Fig. 1). At an absorption at 420 mμ of 0.340, samples were centrifuged and cells resuspended in different lysing solutions. The percentage of reduction in scattering and of release of β-galactosidase and RNA were determined as in Fig. 2 to $\pm$ 5 percent. Cells grown in 20 percent sucrose (in nonfragile form) release less than 1 percent RNA or enzyme and show no reduction of scattering in any of the lysing solutions. T, tris (10 m*M*, *p*H 7.3); TMG, T + 5 m*M* $MgSO_4$.

Lysing solution	Reduction in scattering (%)	Macromolecules released	
		β-Galactosidase (%)	RNA (%)
Tris, 10 m*M*, *p*H 7.3	88	100	88
T + 5 m*M* $MgSO_4$	76	86	66
T + 0.1% sodium deoxycholate	89	100	100
TMG + 0.1% sodium deoxycholate	86	98	81
T + 0.5% sodium deoxycholate	92	100	100
TMG + 0.5% sodium deoxycholate	92	100	100
TMG + 0.5% Brij 58*	77	100	59
TMG + 1.0% Brij 58*	80	95	53

* See (*7*).

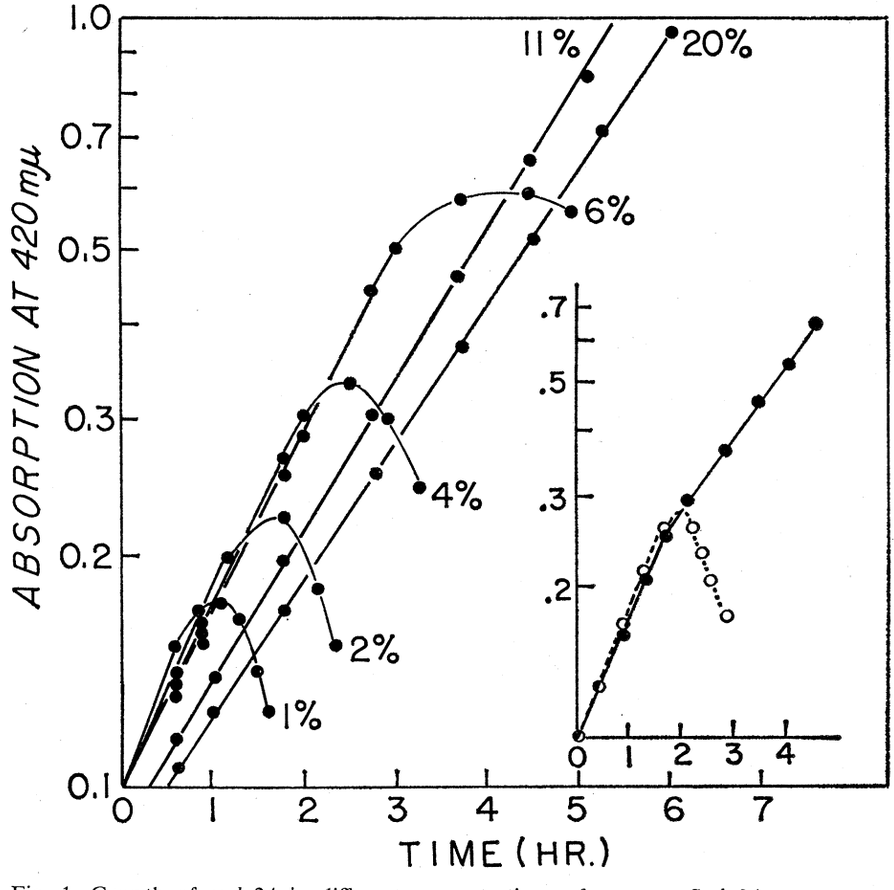

Fig. 1. Growth of *sud* 24 in different concentrations of sucrose. *Sud* 24 was grown at 30°C in a medium containing, per liter, K_2HPO_4, 1.12 g; KH_2PO_4, 0.48 g; $(NH_4)_2SO_4$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.49 g; $FeCl_3 \cdot 5H_2O$, 0.5 mg; glucose, 2 g; 0.4 percent technical grade casamino acids (Difco); and 20 percent sucrose. At 1.5 $\times$ 10^8 cells/ml (zero time), the culture was diluted into fresh medium containing different concentrations of sucrose, as indicated in the graph. The inset shows the growth curve of *sud* 24 in medium containing 4 percent sucrose in the absence (---○---) or the presence (—●—) of 15 percent carbowax 4000.

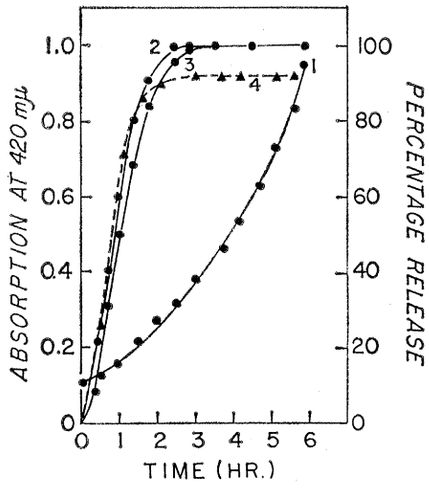

Fig. 2. Growth and fragility of *sud* 24 grown in 4 percent sucrose. *Sud* 24 was transferred at zero time from medium containing a high concentration of sucrose into growth medium containing 4 percent sucrose, 15 percent carbowax 4000, and 0.02 μc/ml of ^{14}C-uracil. Isopropyl-β,D-thiogalactoside ($4 \times 10^{-4}M$) was added at an absorption of the culture of 0.05 to induce the formation of β-galactosidase. At intervals during growth, samples were centrifuged and cells were resuspended in 0.5 percent sodium deoxycholate and tested for the remaining absorption at 420 mμ, β-galactosidase activity (*8*), and for ^{14}C-uracil incorporated in acid-insoluble form. Lysis takes place immediately even at 0°C at cell concentrations up to 5×10^8 per milliliter. A portion of the same suspension in 0.5 percent sodium deoxycholate was centrifuged, and the supernatant was tested for β-galactosidase activity and RNA content to determine the percentage released from the cell. Curve 1, absorption of intact cells at 420 mμ; then, after lysis; curve 2, percentage release of β-galactosidase activity; curve 3, percentage release of ^{14}C-labeled acid-insoluble RNA; and curve 4, percentage decrease of optical density.

index of the release of cellular material, probably because it requires the exit of very large polyribosomes from the cells. Sodium deoxycholate (0.5 percent) has consistently given more complete lysis than the other agents we tried (including 0.01 percent cetyltrimethylammonium bromide, 0.05 percent sodium lauryl sulfate, and those listed in Table 1).

In these fragile cultures, the amount of residual exponential growth at low levels of sucrose (Fig. 1) is roughly proportional to the concentration of sucrose. This residual growth probably cannot be attributed to the utilization of some fraction or impurity of the sucrose for the following reasons. (i) Although growth is more variable, it does occur in 20 percent glucose or in 20 percent α-methylglucoside, a nonmetabolizable analog of glucose; thus, sucrose metabolism is not necessary for the action of sucrose on the mutant. (ii) The same lot of 4 percent sucrose medium in which a culture has stopped growing will repeatedly support the growth of more cells (centrifuged out of high sucrose) to the same limited extent. We infer that sucrose itself does not directly supply a growth factor, but that, in *sud* 24, the synthesis of some component of the cell wall is dependent in an unknown way on the concentration of sucrose in the medium. As the concentration of sucrose is lowered, the rate of growth outstrips the rate of formation of the critical component, and, at about 3 to 5 percent sucrose, a state is reached in which exponential growth can continue, but the cells formed are highly fragile.

Agents like carbowax 4000 can protect these cultures against osmotic lysis as sucrose does but, unlike sucrose, cannot themselves promote the synthesis of the required component. The precise action of sucrose on *sud* 24, therefore, remains an intriguing puzzle; but the mutant itself becomes a useful tool to study fragile structures and rapid processes in bacteria. Until now, only spheroplasts, which grow poorly, do not divide, and frequently can be lysed only incompletely (*5*), provided such fragile forms. We shall report elsewhere (*6*) on the use of *sud* 24 to obtain preparations of intact polyribosomes. It is likely that this and other sucrose-dependent mutants can be used in studies of envelope specification, construction, and function.

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Effect of Temperature on the Life of Soap Bubbles, and Their Solidification at Low Temperature

Abstract. *Soap bubbles (also films on wire frames) have been solidified at low temperatures. At ~ −30°C the bubbles still behave normally, that is, they can be expanded by blowing air into them and contract when the air is let out. At ~ −80°C they become glassy, have very little surface tension, and cannot be blown up any more. At ~ −120°C they become completely solid. No further change is observed by cooling them to 90° or 77°K.*

Soap bubbles have fascinated young and old for over 2000 years, as illustrations on old Etruscan vases demonstrate. Some of the world's outstanding scientists, such as Isaac Newton, in the past, and Lord Rayleigh, J. Willard Gibbs, James Dewar, W. D. Harkins, and I. Langmuir, in our time, have made soap bubbles and their film properties the subject of imaginative research.

In the classics on soap bubbles (see *1*, *2*), I noticed no reference to any attempt to solidify or freeze soap bubbles, or to any extended study of the effect of temperature on their life. Wilke, in 1773 (*1*, p. 132), did see snow flakes settle on soap bubbles, and Pfaff, in 1829 (*1*, p. 140), noticed ice crystal formation in "gossamer leaflets" in soap film.

Owing to the modern development of cryogenics, it was easy to watch the behavior of soap bubbles placed in an ordinary household refrigerator (−11°C) or a deep freezer (−21°C). I observed bubbles (i) hanging from a blow tube in a round flask, (ii) as hemispheres blown over a petri dish, or (iii) as foam in a cylinder. Their average life was five to ten times as long as that of bubbles at room temperature (½ to 1 hour); but their average life was decreased to a few minutes when they were placed in a laboratory drying oven at 100° to 110°C.

My bubble apparatus was simple and is shown in Fig. 1. For most experiments the blow tube, inserted through a rubber stopper, was fixed, by the stopper, in a 1000-cm^3 round-bottom distillation flask, whose side tube was bent upward so that the flask could be submerged easily into a Dewar vessel containing a low-temperature bath. The distillation flask could be evacuated through the side arm, if desired. Most experiments were carried