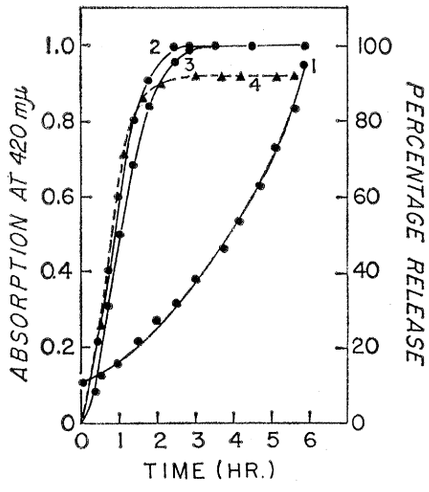


Fig. 2. Growth and fragility of *sud* 24 grown in 4 percent sucrose. *Sud* 24 was transferred at zero time from medium containing a high concentration of sucrose into growth medium containing 4 percent sucrose, 15 percent carbowax 4000, and 0.02 $\mu$c/ml of $^{14}$C-uracil. Isopropyl-$\beta$,D-thiogalactoside ($4 \times 10^{-4}M$) was added at an absorption of the culture of 0.05 to induce the formation of $\beta$-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 m$\mu$, $\beta$-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 $5 \times 10^8$ per milliliter. A portion of the same suspension in 0.5 percent sodium deoxycholate was centrifuged, and the supernatant was tested for $\beta$-galactosidase activity and RNA content to determine the percentage released from the cell. Curve 1, absorption of intact cells at 420 m$\mu$; then, after lysis; curve 2, percentage release of $\beta$-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 $\alpha$-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

out at atmospheric pressure (with the side arm open), but some flasks were evacuated down to 10 torr.

After some preliminary experiments with the classic Plateau solution (*1, 3*), I made bubbles of much longer average life by using Kuehner's solution (*4*) of sodium 9,10-dibromostearate in water and glycerin. Kuehner's solution was further improved by the incorporation of solutions of high-molecular-weight polymers, either inorganic or organic. As inorganic polymers I used the highly viscous solutions of sodium polyphosphates (*5*), with molecular weights up to ~ 15,000,000, made from Graham's, Kurrol's, and Maddrell's salts. As organic polymers, solutions of (i) gelatine and (ii) high-molecular-weight polyalcohols were employed. Furthermore, the monovalent sodium ion (also ammonium and triethanol-ammonium ion) of the Kuehner solution was substituted by the polyvalent (2–5) ions of (i) ethylene diamine, (ii) diethylene triamine, (iii) triethylene tetramine, (iv) tetraethylene pentamine, and (v) 1,3-diaminopropane.

The following observations were made as the temperature of the bath surrounding the bubble was decreased:

1) At −20°C in a deep freezer the bubble (at atmospheric pressure) behaves and shrinks normally.

2) At −30°C (in a Freon-12 bath) the bubble can be expanded normally by blowing air into it slowly.

3) At −78° to −80°C (dry-ice–Freon-12 bath) a Kuehner-solution bubble, after 3½ hours, becomes a very viscous glass; on opening the blow tube to let out air the bubble does not collapse. On the other hand, it does not grow when air is admitted through the blow tube, because it has become porous. At the same temperature a Kuehner solution with my additives is more elastic, and when the air is let out it shows the "parachute" effect, that is, the bubble slowly collapses. If allowed to stand without opening the blow tube, such a bubble develops wrinkles, particularly near the blow tube, and the bubble becomes flabby. These wrinkles can be "ironed out" by admitting air through the blow tube at a slow rate, of ~ 120 $cm^3$ (at normal temperature and pressure) per hour, for a bubble 8 cm in diameter. Rapid admission of air, that is, ~ 1000 $cm^3$ (at normal temperature and pressure) per hour, causes the bubble to break. If some of the bubble solution is not completely drained off, a "polar" ice cap of the frozen solution develops at the bottom of the bubble. (Dry ice or $CO_2$, without the flask, causes collapse of the bubble, owing to the reaction between $CO_2$ and the soap solution.)

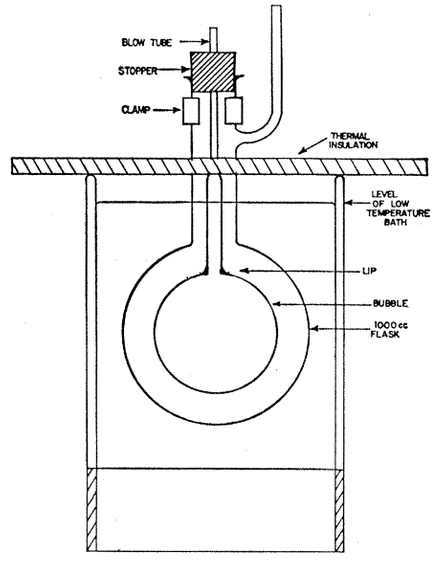


Fig. 1. Apparatus for cooling soap bubbles to any desired temperature level.

4) By cooling slowly from −80° to about −120°C (or 150°K), simply by cooling the Freon-12 bath, freed from chunks of dry ice, with liquid nitrogen, the bubble becomes absolutely rigid and solidifies completely. (Generally about the upper third of the bubble shrinks and develops meridianal wrinkles, while the lower portion remains smooth.) Such a solid bubble is, of course, very fragile, since it has the thinness of the original liquid bubble, unless moisture is permitted to condense on its film. It shows the same beautiful iridescent colors as the liquid bubble. Like glass bubbles, it develops circular cracks if dropped. Further cooling the solid bubble down to 90°K or 77°K produces no obvious visible change. One can go directly from the —78°C Freon bath and immerse the distillation flask, either completely or partially, in a liquid oxygen or nitrogen bath. Then the transition to the solid bubble takes place very rapidly—in ½ to 1 minute. The solid bubble can be gently severed from the blow tube and will float, as a boat, on the liquid air condensed inside the flask by the liquid nitrogen bath.

A bubble blown at room temperature can be suspended directly (without a protecting flask) over liquid oxygen or liquid nitrogen and cooled from the bottom up, at any desired rate. It has been observed that when air is let out of a pure Kuehner-solution bubble, cooled in such a manner, the bubble behaves like a saponin bubble, sagging in the form of a long puckered bag; thus, this is not a peculiar property of saponin but is also shown by fatty acid salt solutions at lower temperatures.

Another variation of cooling experiments is to evacuate the round-bottom flask to produce a bubble in a partial vacuum. A bubble of Kuehner's solution, but with the addition of the highly viscous solution of Kurrol's salt treated with Graham's salt, was produced at 40 torr at room temperature; on cooling by complete immersion of the flask in a −78°C bath for 1 hour and then further cooling to −100°C in a liquid nitrogen-cooled Freon-12 bath during 1 hour, it was possible on occasion to produce a perfect frozen sphere without shrinkage or wrinkles.

Experiments were not confined to spherical bubbles. By cooling a pure Kuehner-solution *foam* in a test tube for 1 hour to −20°C, for 2 hours to −78°C, and then to 90°K, it was possible to solidify most of the foam; when broken with a cold rod it formed beautifully iridescent, gossamer-light platelets.

Soap *films* on a thin copper wire support have been solidified (inside a test tube) by immersion first in a dry ice bath and then in a liquid oxygen bath. Since the solidified films are very fragile, thin wires have to be used for the frame. Rigid frames cause cracks in the film. A tetrahedron thin wire frame, immersed in the pure Kuehner solution, produced a typical *Plateau liquid border*, which could be successfully preserved at low temperatures.

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