The experimental bearings are made of glass to permit viewing of the two-phase film. Inclined mirrors on either side of each bearing provide a full view. Encoding markers on the rotor are sensed optically to monitor speed and to furnish triggering signals for the camera and synchronized stroboscopic lighting.

The journal bearing module operates in the coast-down mode. The drive mechanism is disengaged when the rotor speed reaches 600 rev/min. The camera drive is synchronized with the rotor so that events related to unavoidable variations around the journal surface remain unchanged in successive cinematographic views. Noncontact proximity sensors along two mutually perpendicular axes are used to monitor the radial motion of the rotor.

The cinematographic records showed the following results, which correspond to the three bearing configurations described above:

1) With the balanced rotor, a streamer structure which almost wraps around the full circumference is seen. The appearance fluctuates and repeats in approximately every other frame. This suggests the presence of a one-half rotational rate oscillation. The possibility of such a flow structure was previously postulated (*3*). This general appearance continues as the rotor slows down to about 200 rev/min. The void content of the streamer structure is somewhat reduced at the lower speeds. With the unbalanced rotor, the two-phase morphology changes with speed. At the higher speeds, there is a more pronounced circumferential variation in the flow structure in each frame, but the frame-to-frame fluctuation is less apparent. When the rotor speed falls below about 300 rev/min, the general appearance reverts back to that seen for the balanced rotor.

2) Well-defined void regions are fixed to the three-arc geometry. Occasionally, small isolated voids are seen going around. There are no significant frame-to-frame fluctuations.

3) The general appearance is similar to that in the second case, except that fixed void regions are less extensive and are not divided into three groups.

Data from the proximity sensors remain to be analyzed.

C. H. T. PAN
Department of Mechanical Engineering, Columbia University, New York 10027

RAYMOND L. GAUSE
ANN F. WHITAKER
NASA–Marshall Space Flight Center, Huntsville, Alabama 35812

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2. The lubricants used were SRG-10 superrefined paraffinic oil; Bray 815Z, a vacuum-distilled perfluoroalkyl polyether; Apiezon C, molecularly distilled paraffinic; and a 90:10 blend of four- and five-ring polyphenyl ethers. Finishes of the 440C stainless steel surfaces were ground (roughness 200 to 300 nm, finished by parallel grinding) or polished (roughness 25 to 50 nm, random texture). The surfaces were either clean, prewetted, or barrier film–coated; barrier film was a brush-applied fluoropolymer in solution with perfluorinated cyclic ether per military standard MIL-B-81744.
3. C. H. T. Pan, *J. Lubr. Technol.* **103** (No. 3), 320 (1980).

4. This experiment is a collaborative effort between Columbia University and the Materials Processing Laboratory of NASA–Marshall Space Flight Center (NASA-MSFC). Keith Demorest was another principal investigator when planning for the experiment began; his premature departure is sadly noted. S. F. Murrary and S. Calabrese of Rensselaer Polytechnic Institute contributed significantly to the conceptual development of the experimental approach and to the preliminary feasibility assessment. Final hardware preparation to ensure flight worthiness was capably carried out by technical staff at NASA-MSFC under the leadership of R. Taylor and W. Wall.

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Protein Single Crystal Growth Under Microgravity

Abstract. *The preparation of suitably large protein single crystals is essentially the rate-determining step of protein x-ray structure determinations. Attempts to produce single crystals with two model compounds—β-galactosidase and lysozyme—under conditions of microgravity were successful. Crystals formed by salting out from solutions kept free of convection were 27 and 1000 times larger in volume, respectively, than those produced in the same apparatus but exposed to terrestrial gravitation.*

Solving the three-dimensional molecular structure of proteins by x-ray diffraction analysis helps in understanding their manifold functions (catalysis, transport, and supporting functions). An essential condition for such structural investigations is the availability of sufficiently large (about 1 mm in each dimension) and well-shaped single crystals. Especially disadvantageous for such protein crystal growth is the sudden formation of multiple seeds. Instead of a few large crystals, numerous small crystallites are formed which are useless for x-ray analysis. Experiments have shown that this effect is mainly due to convection, which can be almost completely suppressed by crystallization in gels. However, fragile protein crystals, expecially those growing as needles, break and are destroyed when they touch the tight network of the gel.

It seemed reasonable to look for conditions under which three solutions of different densities ($\rho_B < \rho_P < \rho_S$)—a protein solution (P), a salt solution (S), and a gel-free buffer solution (B)—could be simultaneously and slowly combined without turbulence. Since B is the lightest of these components, such an experiment cannot be carried out by layering the solutions on top of each other. Conditions of microgravity were chosen in order to avoid differences in density ($\rho_B \sim \rho_P \sim \rho_S \sim 0$ g/cm^3). In the experiment setup designed for microgravity, the protein and salt solutions are separated by a sliding device. In a second system of containers the buffer solution is kept. When all the containers have been filled without bubbles, they are closed to the outside by elastic membranes, which allow pressure compensation. When microgravity conditions have been attained, the sliding device is moved without vibration in order to connect the three different containers. Counterdiffusion of P and S across the buffer solution then takes place. The slowly diffusing salt ions cause the protein molecules to crystallize.

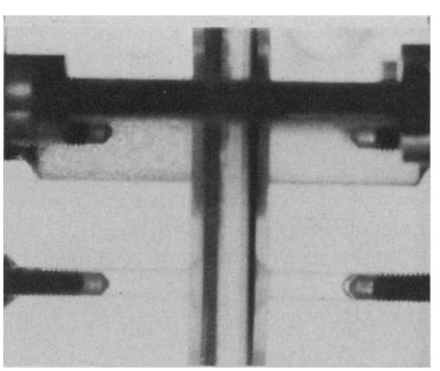

Fig. 1. Protein chamber with large single crysals of lysozyme (left side) and salt chamber (right side).

Since thermal changes could occur during this process and could lead to convection even in the absence of gravitational forces, preliminary short-term experiments (6 minutes of microgravity) were carried out with sounding rockets (European TEXUS program) in order to study the diffusion process with Schlieren optics. The high molecular weight protein β-galactosidase and the salt ammonium sulfate, both dissolved in M/30 phosphate buffer at *p*H 6.4, were used for testing. The experiments under microgravity, in contrast to those under

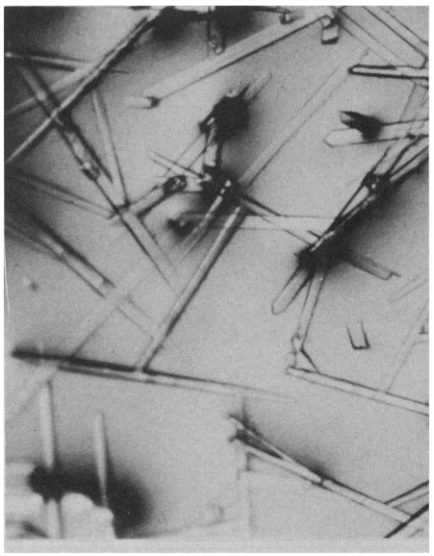

Fig. 2. Single crystals of β-galactosidase produced in Spacelab 1.

terrestrial conditions, showed a strictly laminar diffusion process. Convection and turbulence could be observed only when the payload began to reenter the gravitational sphere of the earth. In addition, an unexpected result was obtained: after only 6 minutes of gravity-free conditions, several single crystals of β-galactosidase formed. The material, examined by microscopy under polarized light, was rod-shaped and averaged approximately 100 μm in length. Because of the short crystallization time the crystals were of inferior quality, but still comparable to those obtained under terrestrial conditions in several days. This increased tendency for crystals to form under microgravity may be due to existing areas of molecular order in the highly concentrated (30 mg/cm^3) protein solutions. It is plausible that under turbulence-free conditions such preordered clusters can readily aggregate further into crystalline material, whereas the strong convection that exists under terrestrial conditions would cause them to break up into smaller particles or even into separate molecules, which would take longer to become reordered and eventually crystallize (*1*).

Considerably longer periods were available for crystallization experiments on the Spacelab 1 flight. Here we used two proteins with widely differing molecular weights in order to determine the extreme limits for future experiments with other proteins of intermediate molecular weight. On Spacelab 1 we used β-galactosidase [β-D-galactoside galactohydrolase (E.C. 3.2.1.23)], molecular weight 465,000; and lysozyme [mucopeptide *N*-acetylmuramoylhydrolase (E.C. 3.2.1.17)], molecular weight 14,307. β-Galactosidase is a key enzyme in genetic investigations and, because of the lack of suitable crystals, its structure is still unknown, whereas the structure of lysozyme was solved 17 years ago (*2*). In order to obtain sufficient material of extreme purity, which is essential for single-crystal growth, we worked out special methods, described in (*3, 4*).

The apparatus used in the Spacelab experiment (*1, 4*) corresponds to that described above. This device operates automatically in order to reduce manual operations by the scientific astronauts as much as possible, and several studies can be carried out in one experiment. The studies cover (i) crystal growth at constant temperatures (20°C) in a stabilizer, (ii) crystal growth in an increasing linear temperature gradient from −4° to 20°C (because the solubility of many proteins decreases with increasing temperature), (iii) the use of different protein concentrations, and (iv) the influence of wide and narrow diffusion fronts.

The device consisted of two acrylic blocks, each of which contained four samples for crystallization. To suppress protease activity which may cause protein degradation, the blocks were kept near 0°C in a special Dewar storage container, kept cold by melting ice without any external energy supply. They were transported at 0°C into the spacecraft, where, after conditions of microgravity were reached, they were removed from the cooling container and placed manually in the apparatus designed for the crystallization experiment. Operation of a switch triggered the machinery, set at the different initial temperatures (20°C in the stabilizer, −4°C in the freezer), and started the process of diffusion and crystallization.

After the space mission was terminated and the sample containers were retrieved, the events during spaceflight were analyzed and the following preliminary findings were made.

1) Protein crystallization takes place in the protein chamber because of the low velocity of diffusion of the protein molecules (see left side in Fig. 1).

2) Single crystals are significantly larger than those obtained in the same apparatus under terrestrial conditions. β-Galactosidase shows a 27-fold increase and lysosome a 1000-fold increase in volume (Figs. 2 and 3).

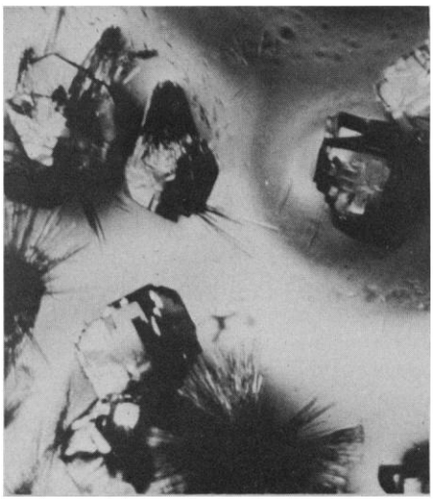

Fig. 3. Single crystals of lysozyme produced in Spacelab 1.

3) The crystalline material is well shaped and of good quality, as determined by examinations under polarized light.

4) Wide fronts of diffusion favor the formation of large crystals.

5) Applying an increasing temperature gradient improves crystallization of β-galactosidase.

6) Perfect habit is found with crystals grown free, floating out of the solution without touching the wall.

Further investigations and x-ray tests are in progress. The question of whether this procedure can be applied to the crystallization of other types of proteins can be answered only after further examination.

WALTER LITTKE
CHRISTINA JOHN
Chemisches Laboratorium der Universität Freiburg, D-7800 Freiburg, Federal Republic of Germany

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5. We thank the Bundesministerium für Forschung and Technologie, Bonn, for financial support.

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