CRYOSTAT
18-IML-1

DARA, GmbH Germany

Hardware

The Facility

The CRYOSTAT is an autonomously working rack-mounted equipment (Fig. 1). It provides two thermostat chambers, independently controlled by a processor via on/off switching of the current through peltier elements.

The temperature profiles of the freezer and stabilizer are subdivided in a common number of steps, each one with a preprogrammable temperature gradient or at constant temperature. Core parameters can be reprogrammed by crew interaction in case of rescheduling the CRYOSTAT operation time due to changed mission requirements or contingency.

Actions of the CRYOSTAT (e.g., opening the slide), the steps, actual temperature of the thermostat chambers, experiment time, and the housekeeping data are recorded on a built-in RAM and a tape.

In each thermostat chamber a specific sample container can be inserted which consists of a transparent Plexiglas block accommodating seven crystallization experiments.

The Sample Container

A schematic cross section of a sample container (Figs. 2a and 2b) demonstrates the operational principle:

In the non-operation mode each of the cylindrical crystallization volumes arranged in parallel in the sample container is divided by a slide to separate the protein and the salt solutions. Sealing of the reservoirs against each other and against the other experiments is achieved by a set of O-rings. For initiating the crystallization process the slide has to be pushed in by a slide drive mechanism and assigned holes in the slide filled with buffer solution will establish the contact between these solutions.

The volumes per experiment for protein, salt and buffer solutions are 0.57 ml, 0.84 ml, and 0.67 ml, respectively.

The Operation

The Cryostat Stowage Container, a dewar (Fig. 3), containing the two sample containers is loaded in the middeck about 17 hours before launch. Once in space, the crew activates the CRYOSTAT. After facility conditioning the samples are inserted in the thermostat chambers. The crystallization process starts automatically if the initial temperature for the samples is reached. The operational/temperature profile for the IML-1 samples is shown in Fig. 4. When the experiment ends, the crew remove the samples, put them back in the Middeck stowage for early retrieval, and deactivate the facility.

After the flight, the crystals are analyzed by x-ray crystallography and compared to terrestrially grown crystals.

Technical Data of CRYOSTAT:

Operating Temperature Range:	
Stabilizer:	+ 15 °C to + 25 °C
Freezer:	- 4 °C to + 20 °C
Accuracy:	± 0.5 °C
Chamber Volumes for Sample Container	54 mm x 54 mm x 182 mm
Data Storage Capacity	240 kB in buffered RAM 10 MB on tape recorder
Cryostat Stowage Container, Holding Time (20 °C amb. temp.)	65 hours

---

The Experiments

Single Crystal Growth of Beta-Galactosidase and Beta-Galactosidase/Inhibitor-Complex

Principal Investigator:

Dr. W. Littke, University of Freiburg, Chemical Laboratories, Freiburg, Germany

For this investigation the CRYOSTAT is used in the freezer mode. Temperature starts at -4 °C and gradually increases to 20 °C. The total concentration of the salt (ammonium sulfate) in each chamber system is constant.

The protein to be crystallized β-galactosidase and β-galactosidase/inhibitor-complex, respectively.

β-galactosidase is an enzyme that hydrolyzes lactose (glucose-4-β-D-galactoside), and is found in intestines of babies and baby animals as well as in E. coli. It is the key enzyme of modern genetics and therefore one would like to determine the three-dimensional molecular structure of the compound to find out the interaction mechanism between function and structure.

The high molecular substance (465.000 D) is the first protein of space history crystallized in space in 1983 on Spacelab 1 using the CRYOSTAT. The crystals were several times larger and more perfect than those produced under terrestrial conditions. Because of limited quantity available, x-ray studies with the crystals could not be finished.

Crystal Growth of the Electrogenic Membrane Protein Bacteriorhodospin

Principal Investigator:

Professor Dr. G. Wagner, University of Giessen, Botanical Institute 1, Giessen, Germany

This experiment uses the stabilizer mode. In this mode the temperature remains stable at 20 °C, but the concentrations of the salt and the buffer solutions are varied from sample to sample, so investigators can determine which concentration promotes the growth of better, larger crystals.

The protein to be crystallized is bacteriorhodospin, a well-known membrane protein that converts light energy to voltages in the membranes of photosynthetic archaebacteria.

Microbiologists are interested in this system because bacteriorhodopsin represents an almost ideal system to study light-energy-driven vectorial membrane transport developed in Earth's early environment.

Many ground-based experiments have been done with bacteriorhodopsin, that forms two-dimensional crystals on high salt concentrations. However, resolution of the three-dimensional structure, which will help biologists understand how bacteriorhodopsin works, depends on the availability of isometrically large, highly ordered crystals. Hitherto, high-quality crystals have not been grown under terrestrial conditions.

Crystallization of Proteins and Viruses in Microgravity by Liquid-Liquid Diffusion

Principal Investigator:

Dr. A. McPherson, Department of Biochemistry, University of California at Riverside, USA,

Canavalin:

Canavalin is a representative of a highly homologous class of plant proteins classically known as vicillins. These are the major storage proteins of leguminous seeds such as kidney beans, green beans, garden peas, and most other edible beans and seeds. Thus, this plant seed protein is among the largest sources of nutritional protein available to man for his own consumption and that of his agriculturally important animals. It is a crucial component of the world's diet, particularly in the developing countries. Its improvement by genetic means to enhance its nutritional properties is, therefore, a major objective of protein engineering as applied to the agricultural sphere. Success in enhancing its nutritional properties, its viability and its abundance could contribute substantially to the alleviation of world hunger and famine.

The protein itself is of appreciable interest as well to the biochemist and molecular biologist. Its structure at the atomic level has now been determined by three-dimensional x-ray diffraction analysis, and the gene coding for its expression has been cloned. Thus, all the elements are in place for the systematic and rational application of automated computer graphics analysis, site directed mutagenesis and recombinant DNA technology to the modification of its physical and chemical character. It represents one of the few clear examples where this is true.

The protein is a trimeric molecule of 150,000 daltons, composed of three identical subunits. X-ray and genetic analyses have shown the 50,000 dalton subunit to be internally redundant in terms of both amino acid sequence and three-dimensional structure, thus, the gene which codes for this protein must be a tandem duplicate. This is one of the few proven examples of such a case. The oligomer possesses a perfect threefold axis of symmetry relating it subunits and each subunit, in turn, contains an internal pseudo dyad axis of symmetry.

The protein, canavalin, can be isolated in very large amounts from Jack Beans (Canavalis ensiformis), a useful cattle feed, by traditional biochemical techniques and is crystallized from

1% NaCl buffered with phosphate at pH 7.2. It was first isolated by the famous biochemist, J. B. Sumner, in 1917 and crystallized by him in 1934. It was, in fact, one of the earlist proteins ever crystallized.

Satellite Tobacco Mosaic Virus:

Satellite tobacco mosaic virus (STMV) is a T=1 icosahedral virus that, along with its master virus tobacco mosaic virus, (TMV), infects tobacco and a variety of other plants. STMV is one of the few known cases of a spherical satellite virus that requires coinfection by a fillimentous virus (TMV). STMV has a molecular weight of about 1.5×10^6 daltons and is composed of 60 copies of a coat protein of molecular weight 17,500 daltons each and a single stranded RNA genome of 1066 nucleotides. The genome codes only for the coat protein, no other product is made.

STMV is the smallest virus ever crystallized and it is under active study by x-ray diffraction analysis to determine its detailed three-dimensional atomic structure. Crystals can be reproducibly grown from ammonium sulfate solutions, from polythylene glycol, and over a broad range of pH values.

Because STMV is such a large particle it offers a number of unique advantages for the study of macromolecular crystal growth. It is the subject of quasi elastic light scattering investigations to delineate nucleation mechanisms, and it provides crystals ideally suited for electon microscopy studies as well.

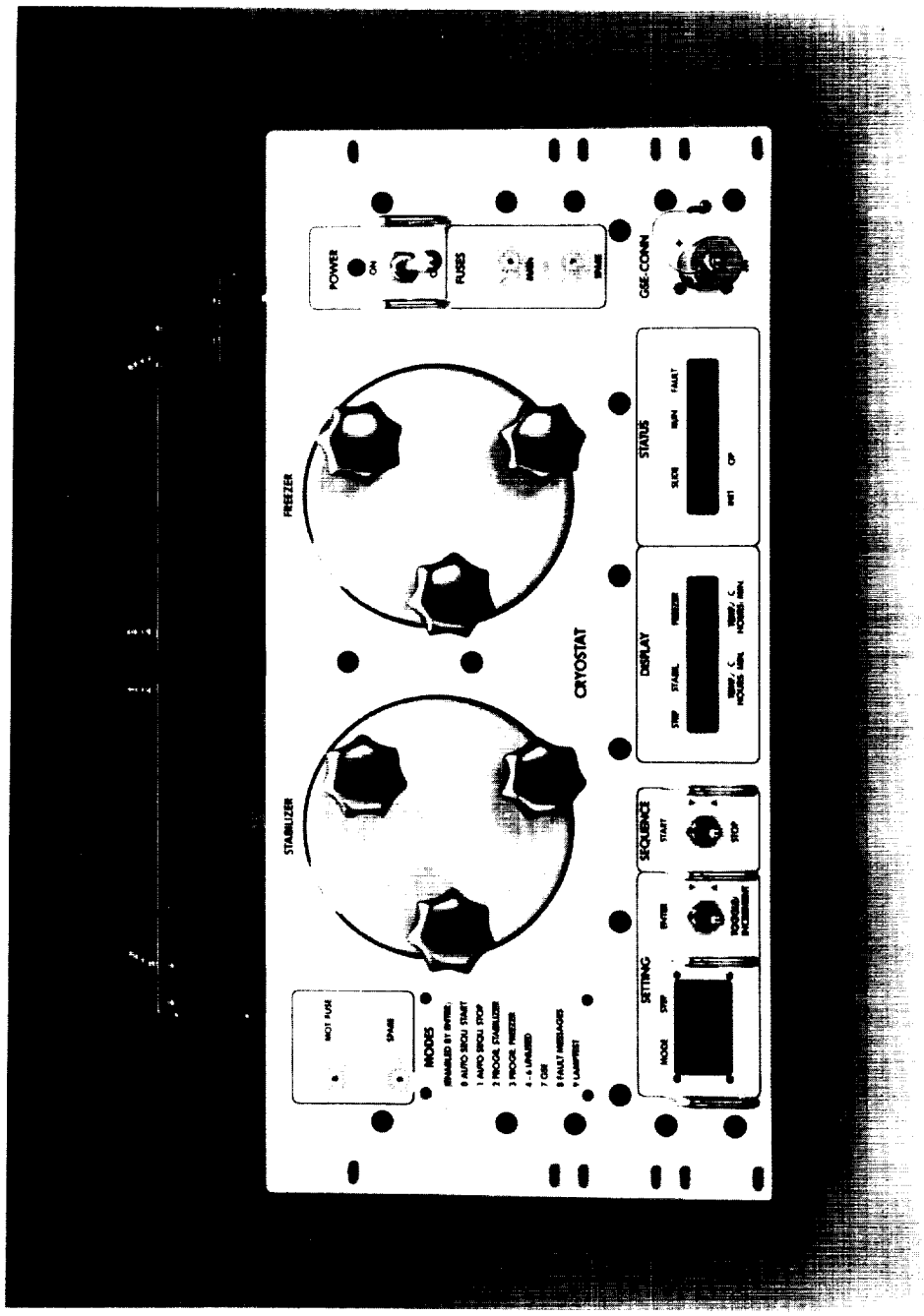

Figure 1. Cryostat front panel.

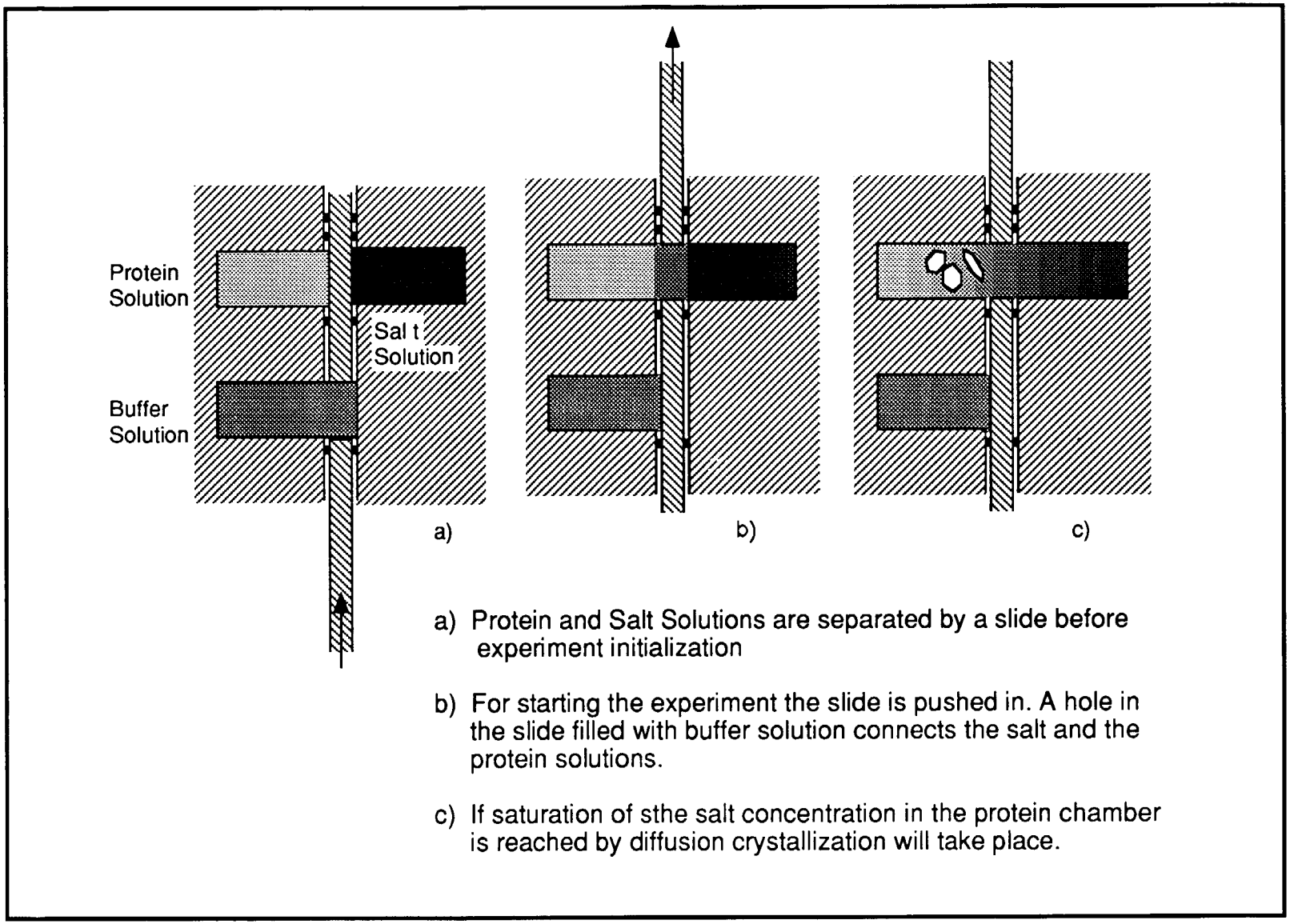

Figure 2a. Cryostat crystallization principle.

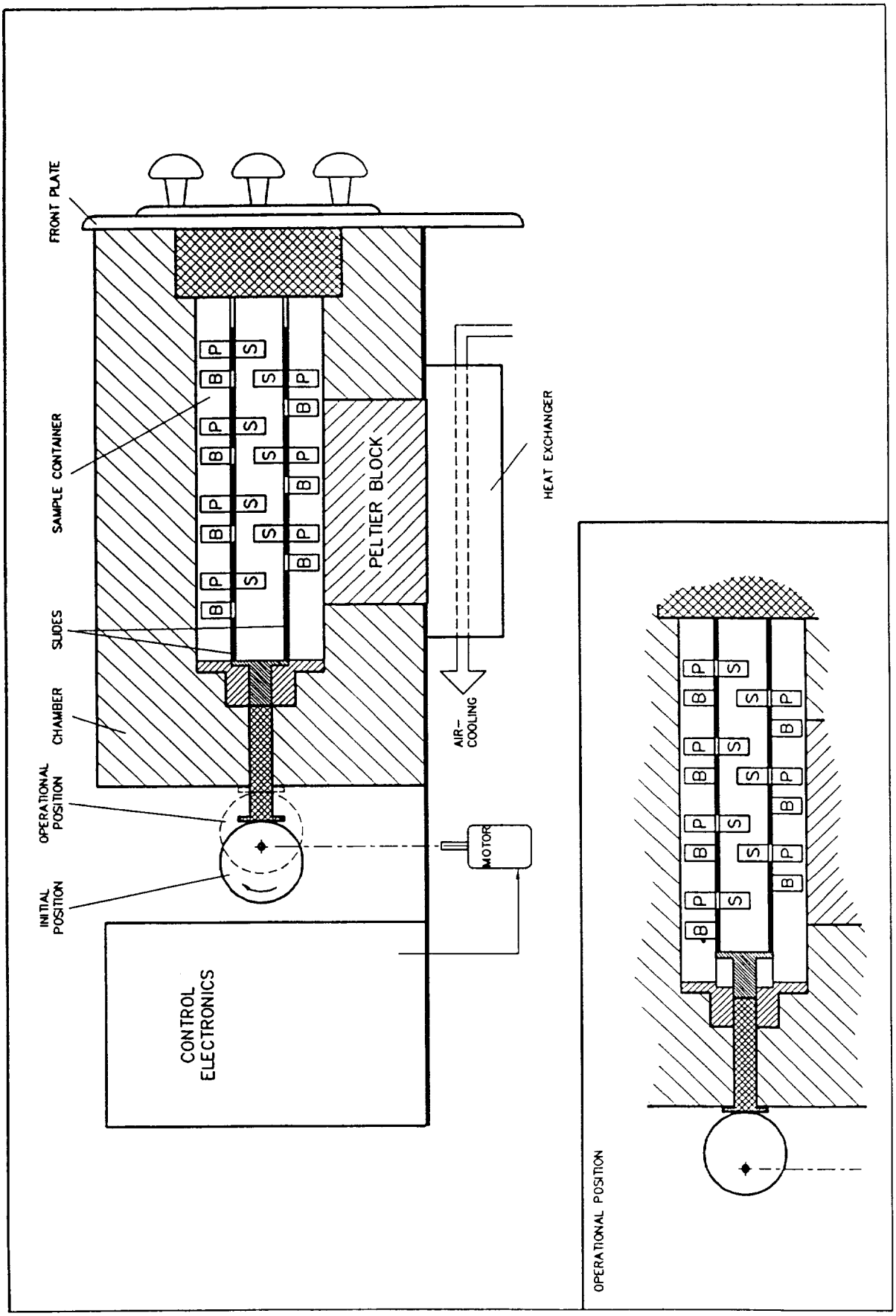

Figure 2b. Cryostat operating schematic.

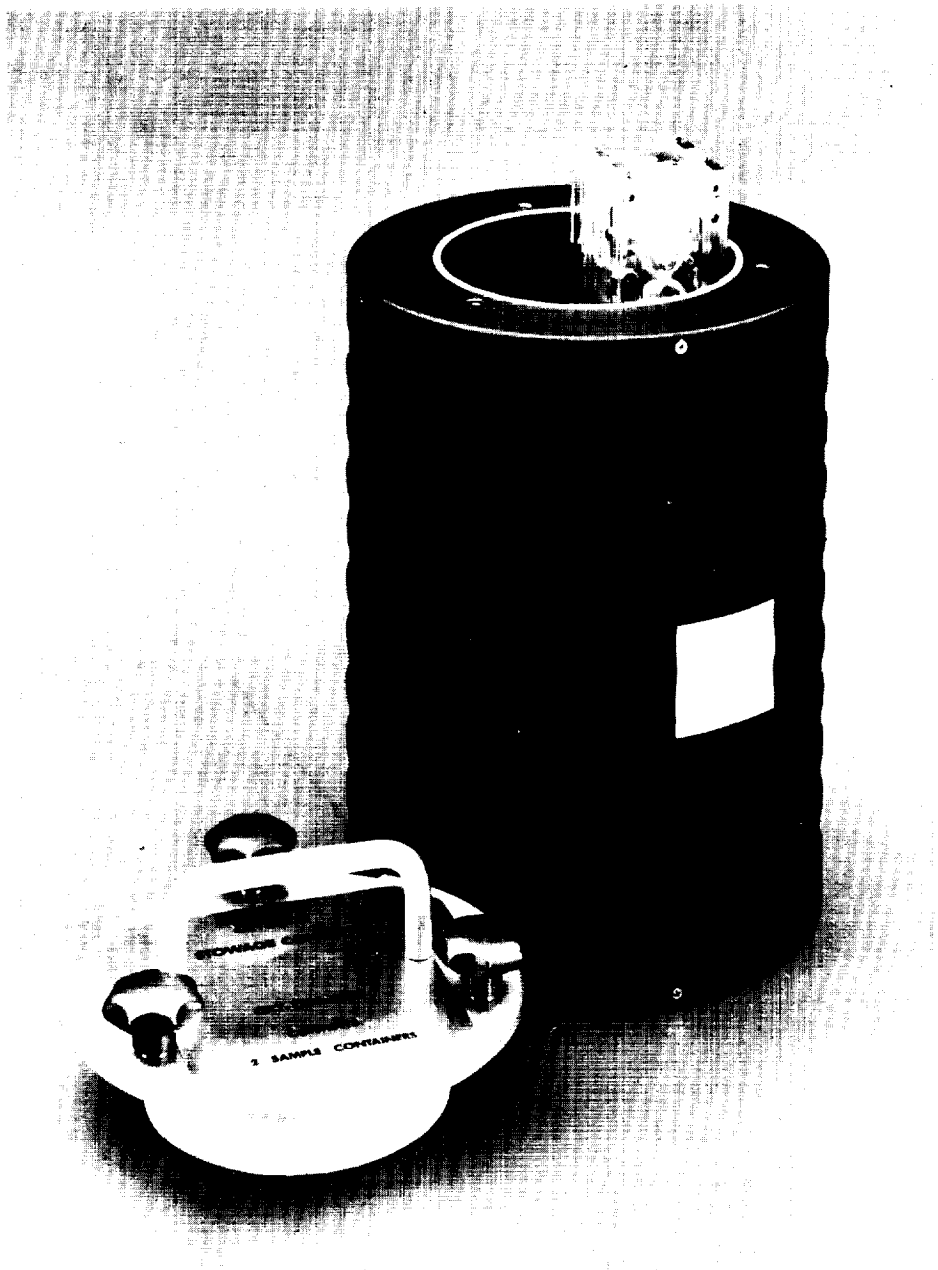

Figure 3. Cryostat sample stowage container.

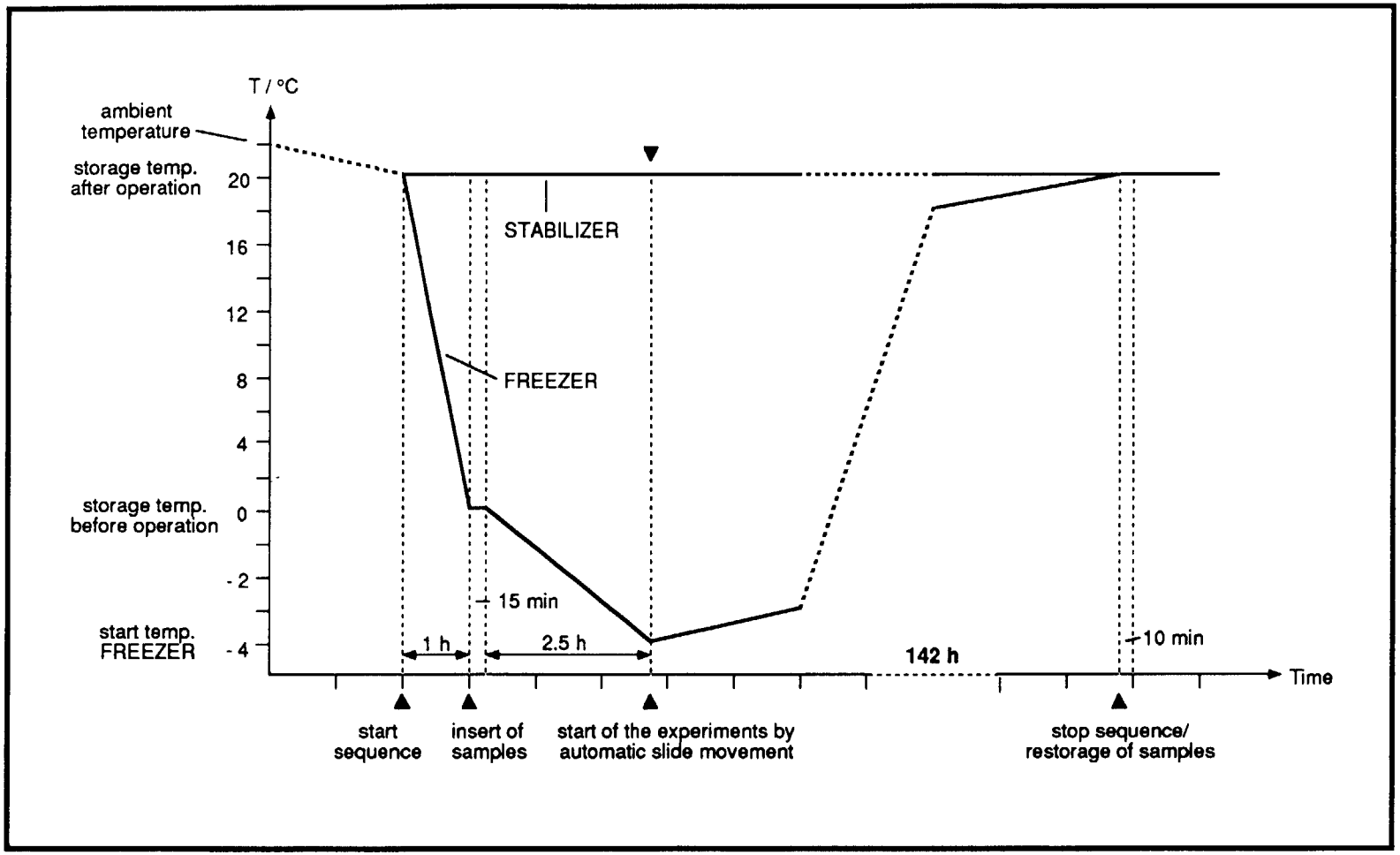

Figure 4. Cryostat experiment temperature/time profile.