

PROTEIN CRYSTAL GROWTH

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Proteins (enzymes, hormones, immunoglobulins, and numerous other types) account for 50% or more of the dry weight of most living systems and play a crucial role in virtually all biological processes. Since the specific functions of essentially all biological molecules are determined by their three-dimensional structures, it is obvious that a detailed understanding of the structural makeup of a protein is essential to any systematic research pertaining to it. At the present time, protein crystallography has no substitute: it is the only technique available for elucidating the atomic arrangements within complicated biological molecules.

Most macromolecules are extremely difficult to crystallize, and many otherwise exciting and promising projects have terminated at the crystal growth stage. Single crystals that have dimensions of 0.2-1.0 mm on a side are generally required for x-ray crystallographic analyses of macromolecular structures and much larger crystals are required for neutron diffraction analyses. Proteins and other biological macromolecules often yield small micro-crystals readily, but it might then take several years of trial and error experimentation before these micro-crystals can be induced to grow large enough for a complete structural analysis. Even when large crystals are obtained, the crystals of essentially all biological macromolecules diffract rather poorly due to internal disorder. Thus, there is a pressing need to better understand protein crystal growth, and to develop new techniques that can be used to enhance the size and quality of protein crystals.

In principle, there are several aspects of microgravity that might be exploited to enhance protein crystal growth. According to theoretical considerations and experiment results, the major factor that might be expected to alter crystal growth processes in space is the elimination of density-driven convective flow. Convection in solution growth is caused by density gradients that occur when solute is depleted from the solution at the growing crystal surfaces. The density-dependent convection might be expected to affect protein crystal growth from aqueous solutions in several different ways. Convection will force solution to flow past the crystal, thus bringing material to the growing crystal surfaces at a rate that is significantly different from the steady-state diffusion rate that would be predominant in quiescent solutions. The flow patterns may generate significant variation in concentration at different parts of a crystal, thus leading to non-uniform growth rates. Also, convection may lead to significant physical stirring of growth solutions; in general, it is expected that such stirring effects might alter nucleation in growth processes.

Another factor that can be readily controlled in the absence of gravity is the sedimentation of growing crystals in a gravitational field. When a protein crystal grows from aqueous solution on Earth, it generally migrates to the top or the bottom of the crystallization vessel (depending on whether its density is greater or less than the density of the solution). Therefore, protein crystals often grow from solution at an interface where all sides of the crystal are not equally accessible to the crystallizing solution. (In most cases, sedimentation causes proteins to crystallize as fused masses that contain highly disordered crystalline arrays.) Under microgravity conditions, it is expected that protein crystals will not display this tendency to migrate away from initial nucleation sites, and can thus grow in isotonic environments, forming discrete, independent nucleation in sites.

Another potential advantage of microgravity for protein crystal growth is the option of doing containerless crystal growth. Contacts with vessel walls often lead to heterogeneous nucleation in crystal growth solutions. In the microgravity environment, it may be possible to form stable spherical droplets of crystallizing materials, which might be suspended by acoustical levitation or other methods. It is definitely possible to form relatively large stable droplets of protein solutions by extruding solutions from a pipette or a syringe; thus, protein crystals might be grown under microgravity conditions in relative large droplets adhering to syringe tips, without the extensive wall effects that generally accompany crystallization experiments on Earth.

As a result of the above theories and facts, one can readily understand why the microgravity environment established by Earth-orbiting vehicles is perceived to offer unique opportunities for the protein crystallographer. This perception led to the establishment of the Protein Crystal Growth in a Microgravity Environment (PCG/ME) project that continues today under NASA sponsorship. This project has advanced from simple hand-held devices (containing only a few protein solutions) to a more complex system involving 60 individual protein experiments in a thermally-conditioned environment. The results of experiments already performed during STS missions have in many cases resulted in protein crystals being grown that are significantly larger and more structurally correct than the best specimens produced on Earth. Thus, the near-term objective of the PCG/ME project is to continue to improve the techniques, procedures, and hardware systems used to grow protein crystals in Earth orbit. A large number of industrial guest investigators and co-investigators are involved in the project and multiple flight opportunities are obviously required to accomplish these objectives.

During the mission, protein crystals will be grown by vapor diffusion which is a technique that uses the diffusion of water vapor to establish equilibrium between protein solutions and more concentrated reservoir solutions. The protein and precipitant solutions will be contained separately in double-barrelled syringes located within small experiment chambers. The experiment chambers will also contain absorbent reservoirs saturated with precipitant solutions at higher concentrations.

The PCG payload flying on SL-J will include three vapor diffusion trays (VDAs) contained in a Refrigerator/Incubator Module (R/IM) at 22 °C (Figure 1). Each VDA tray (Figure 2) will contain 20 experiment chambers, each containing a double-barrelled syringe and a containment plug. Each syringe is made of polysulfone and is operated by a ganging mechanism. Prior to launch, the syringes will be loaded with a maximum of 40 µl of protein solution in one barrel and a maximum of 40 µl of precipitant solution in the other barrel, and the absorbent reservoir will be saturated with precipitant solution. The syringes will then be installed in the experiment chambers and capped by containment plugs for launch. One VDA tray will be modified to accommodate seeding of the protein droplets with Seed Insertion Devices (SIDs). Seeding is a technique used to initiate and enhance crystal growth by the introduction of seed crystals into equilibrated protein droplets. During the pre-flight loading procedure, a few protein seed crystals will be loaded into each SID, and then the SIDs will be stowed in the PCG equipment locker.

Shortly after achieving orbit, a crew member will activate the PCG experiments. A handwheel will be attached to a ganging mechanism that retracts the containment plugs from the double-barrelled syringes. Next, the handwheel will be relocated on the tray to operate the syringe ganging mechanism, resulting in the simultaneous movement of the pistons attached to

each syringe. This will force the protein and precipitant solutions from their respective barrels, causing the two solutions to mix as they form a droplet on the tip of each syringe. The pistons will be retracted and extended through several cycles to ensure mixing of the two solutions. At a prescribed time, shortly after PCG activation, the SIDs will be inserted into the appropriate chambers and the seed crystals injected into the protein droplets.

After activation, each protein droplet is surrounded by a saturated atmosphere that is in contact with a reservoir containing a precipitant solution. Water vapor will gradually move from the droplet to the reservoir because the precipitant concentration in the reservoir is higher than that in the drop. This diffusion of water out of the drop causes the concentrations of protein and precipitant to increase in the drop. When appropriate concentrations are reached in the drop, the protein molecules will begin to nucleate, causing crystals to form in the droplet.

Prior to landing, a crew member will deactivate the payload by first turning the hand-wheel to draw the droplets back into the syringes and then plugging the syringe tips with the containment plugs to protect the crystals during landing.

Protein crystals produced by experiments such as these enable investigators to determine each protein's three-dimensional structure, thus leading to new information about the structure and function of protein molecules. Previous PCG experiments have produced many protein crystals, that once analyzed by x-ray crystallography, were found to be larger and more perfect than their ground-based counterparts. Potential applications of the structural information gained from these protein crystals include treatment of organ transplants and HIV infection, production of dietary protein for human and domestic animals, and transfer of genetic materials.

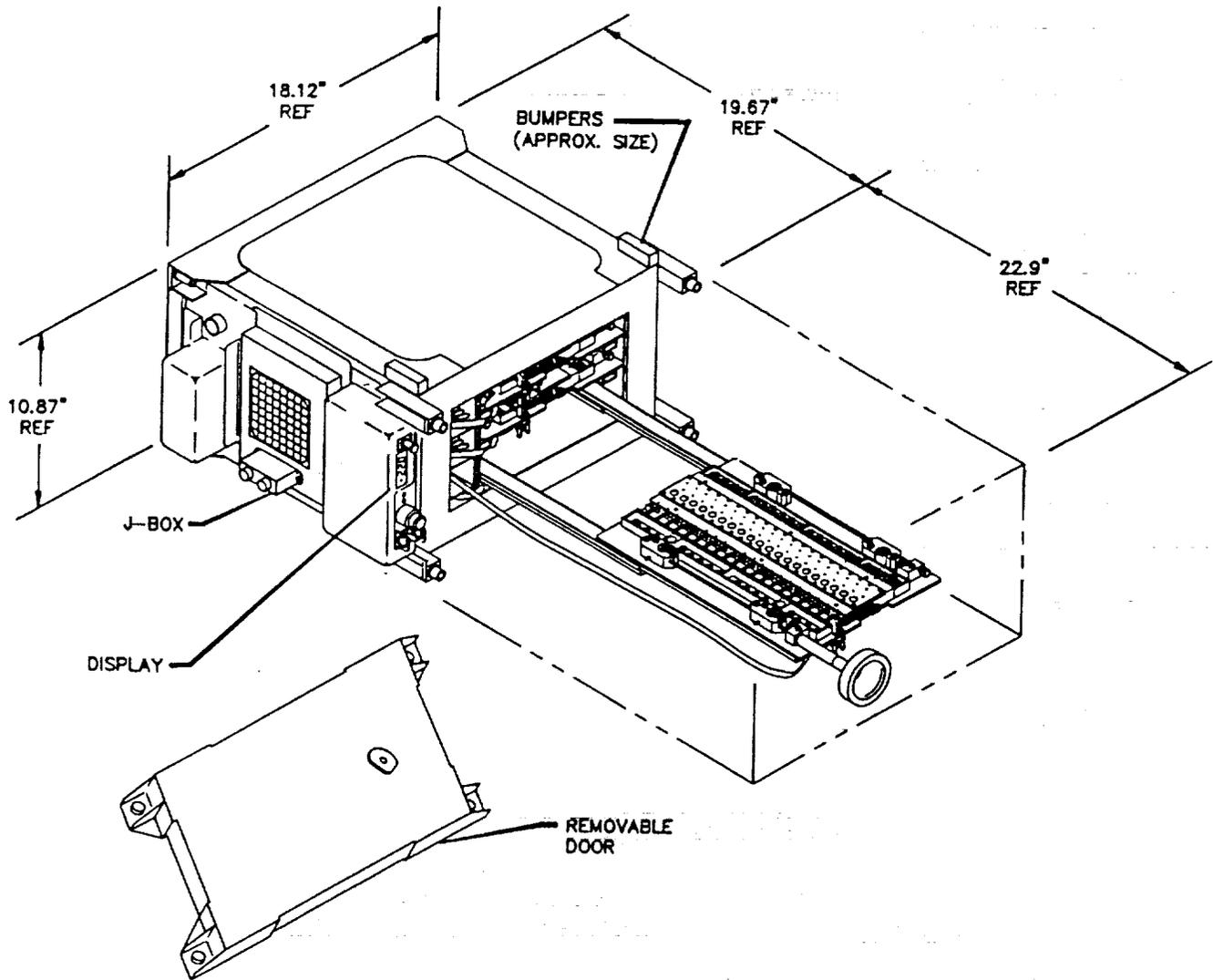


Figure 1. Refrigerator/Incubator Module (R/IM).

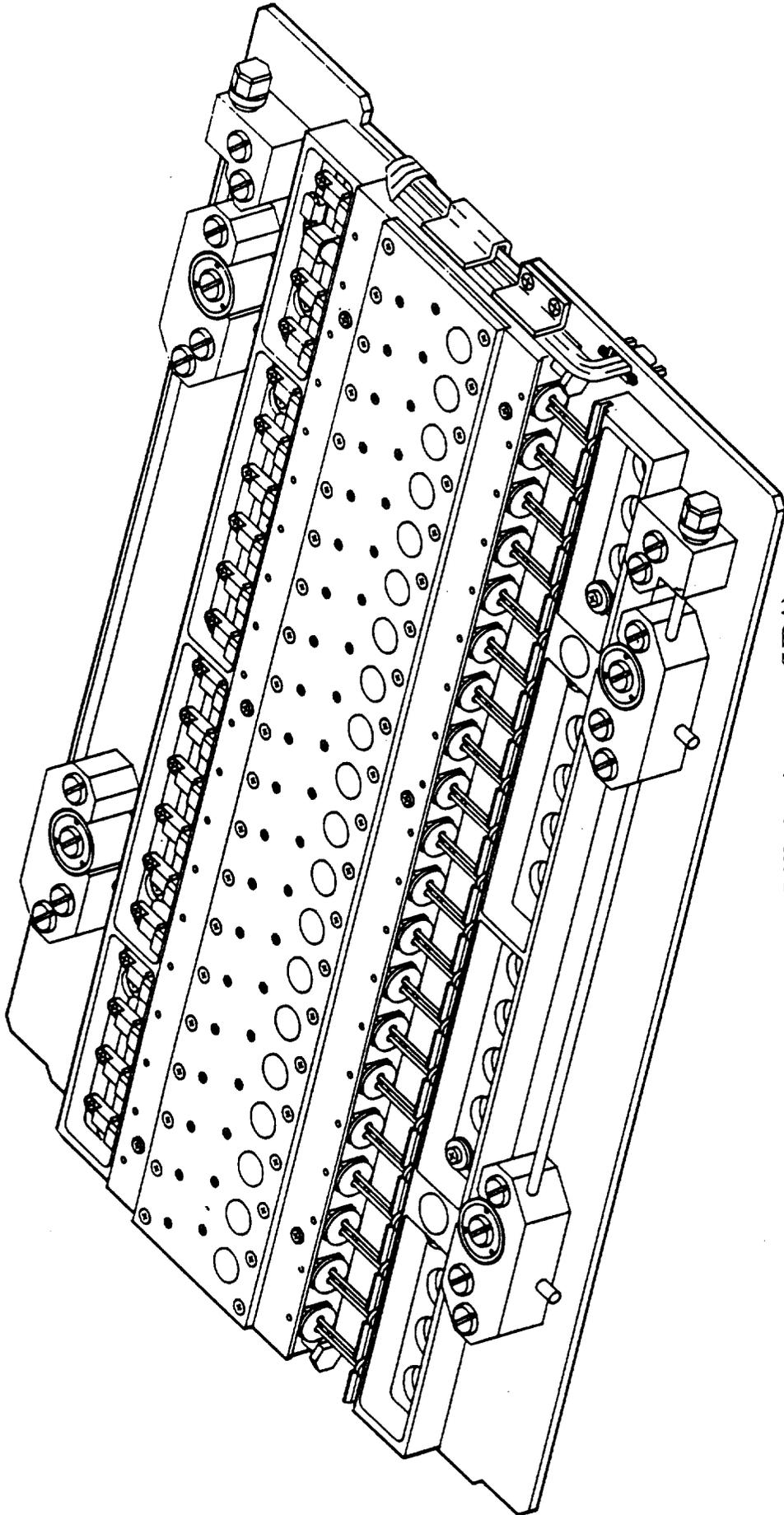
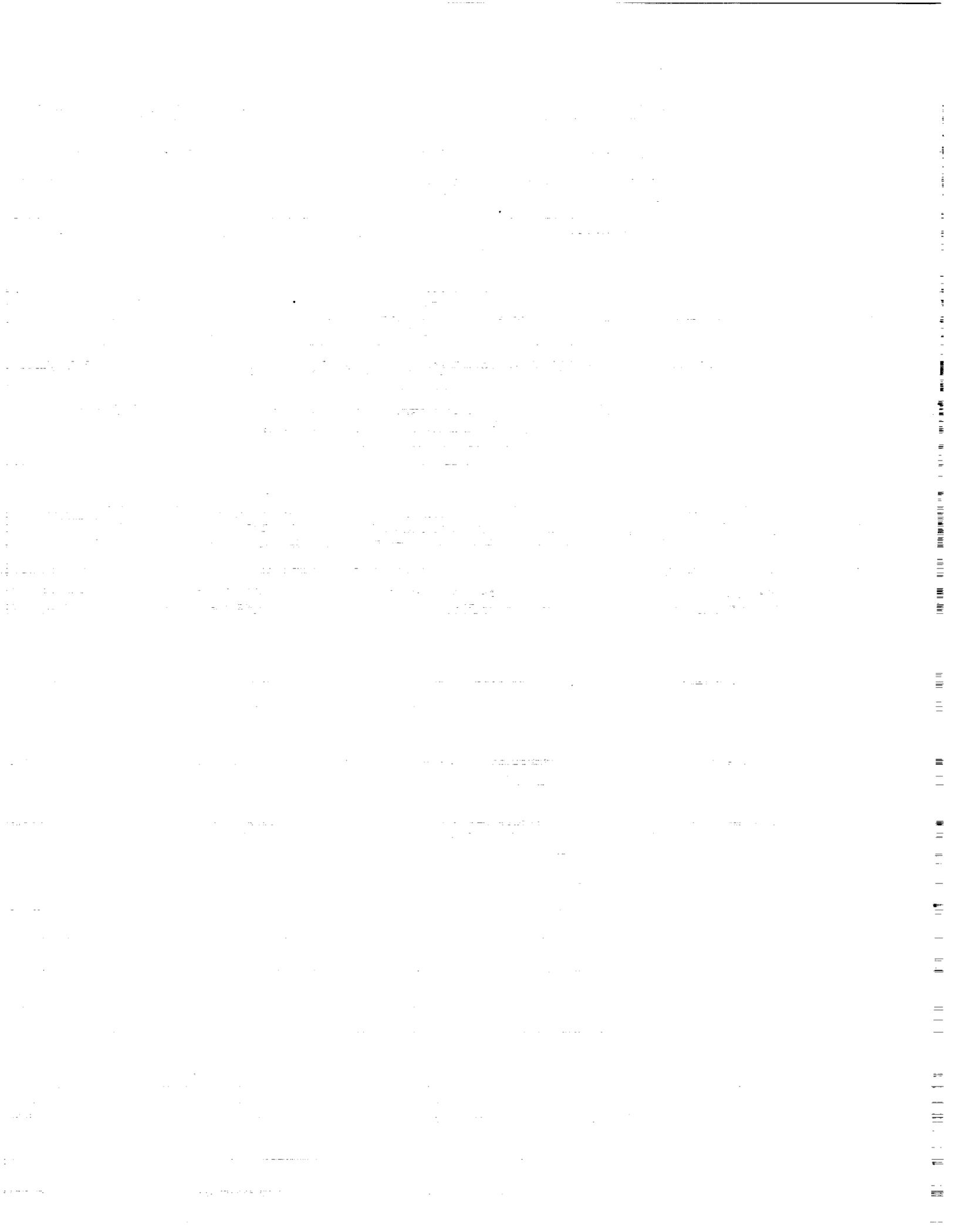


Figure 2. Vapor Diffusion Apparatus (VDA).



**SECTION II
LIFE SCIENCES**

