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Table of Contents

Biotechnology in Space Laboratories

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The advent of the Space Shuttle and of the Spacelab will open new perspectives to biotechnology in space. The objectives of this review are: a) to present an overview on the technological and scientific aspects of biological experiments performed on the past US and Soviet space missions, b) to describe the facilities offered by Spacelab in the future, c) to give practical information on the requirements of flight hardware and on the limits in weight, energy and crew-time. Experiments on Apollo and on the Apollo-Soyuz Test Project have shown that the weightless environment offers advantageous conditions to the processing of mammalian cells. Soviet investigations demonstrated that artificial gravity attenuates some of the disturbing effects of spaceflight observed on animals and plants. An extensive program of preliminary studies should precede large-scale biotechnological applications: Suitable hardware has to be developed in collaboration with ESA and NASA, biological objects should be selected as potential candidates for bioprocessing in space. The preparation of investigations in space should be accompanied by ground high-g simulations in centrifuges and by compensating gravity in clinostats. The exploitation of space resources and the establishment of space colonies is becoming a realistic goal for the next decades.

1 Introduction

Over twenty years of manned spaceflights have shown that man can survive and work in space for a prolonged period of time (more than 6 months). The main objectives of the past missions were to show that man can adapt himself to the new environment and to explore the solar system. Astronomy and astrophysics were the disciplines which most profited from the scientific space program. In comparison, facilities for life sciences were less sophisticated and were flown on a limited number of missions.

With the advent of the Space Shuttle and of the Spacelab we are at the beginning of a new era in space technology: Time has come for the exploitation of space resources.

It is known that weightlessness provides favorable conditions for the production of high-quality materials like metal alloys and glasses. Similarly, preliminary experiments indicate that purification of specialized human and animal cells is sometimes easier to perform in space than in ground laboratories. Therefore, material sciences and bioprocessing are becoming new important disciplines in space sciences. An extensive program of research in space biology will start as soon as Spacelab becomes operational. A basic study on the survival and adaptation of living systems to the space environment has to be performed in parallel with applied research. Every biological experiment in space is a technological challenge since flight hardware must comply with the strict safety and engineering specifications set by the space agencies on the one hand and with the biological requirements of living systems on the other.

The objective of this review are:

- To give a summary of the results and describe the equipment of experiments in space biology performed on past missions.
- To discuss the technological aspects of the preparation and execution of life science projects in space. In particular, we describe our own experiments to be performed on Spacelab;
- to draw the attention of the reader to data and observations often not easily available;
- to stimulate the scientific community to participate in the activities in space taking profit of the facilities offered by the Spacelab and other space stations;
- to analyze the potential benefits of space research;
- to describe systems for high- and low-g ground simulations, and our observations with animal cells under altered g conditions.

Here we discuss mainly experiments and technology dedicated to the study of the behavior in space of microorganisms and animal cells cultured in vitro. We will not discuss equipment for biomedical research, nor for growing plants in space. However, in Section 2 a we will give a survey of the major achievements of life science experiments in space.

Valuable results were obtained on automatic satellites. Unfortunately, complicated mechanisms are often subject to failures. Only the constant presence of man as an intelligent observer and operator can guarantee a critical evaluation of the experiment and a proper function of the equipment. Finally, experiments in space should be, whenever possible, preceded by ground simulation at high and low-g. We will describe a number of interesting results obtained by this approach.

2 Biology in Space

This section presents an outline of the topics belonging to the field of space biology and of their scientific background. The past and the future of space biology have been outlined in a brochure edited recently by Bjursted [1]. Although we will not discuss the technology of growing plants in space laboratories, it must be pointed out that plants are ideal objects for the study of gravitational effects since plant growth is regulated by well identified gravity receptor cells (statocytes) carrying gravity-sensitive organelles (statoliths).

2.1 Man in Space

The primary question at the beginning of the astronautic era was whether humans can survive at all for a prolonged period of time in space. This question was associated with the development of a life-supporting system (energy, atmosphere and waste disposal) capable of providing adequate living conditions to the crewmen. We can regard this phase as completed although technology will progress toward better and better solutions (see Sect. 9).

The physiological problems of man were identified already at the early times of astronautic and were sharply focused after the recent long-duration Soviet flights on the Salyut-6 station. A comprehensive discussion of adaptation of man in space is given in Ref. [2]. A brief summary is presented here.

a) The space motion sickness involves mainly the vestibular apparatus. Its occurrence is unpredictable and cannot be detected by ground simulations. It may be accompanied by serious symptoms of indisposition which can prevent astronauts from performing flight operations.

b) Cardiovascular changes: within few hours in weightlessness, approximately 2000 ml of body fluids (plasma and interstitial liquids) are shifted from the lower to the upper parts of the body. This effect does not cause serious inconveniences.

c) Degradation of bone material and muscle atrophy. These symptoms seem to be in part irreversible. Intense fitness training inflight does not prevent completely the diseases.

d) Hematological and immunological changes: A relevant loss of red blood cells, hemoglobin and plasma volume are observed after every mission. Lymphocyte reactivity is often reduced after spaceflight.

We will discuss here in more detail only those aspects of the adaptation of man in space which can be investigated by biotechnological applications such as *in vitro* simulations of cellular events.

Interesting examples are the immunological and hematological systems: Reduction of red blood cell mass (2—21%) and of hemoglobin mass (12—33%) is generally observed after the US and Soviet space mission. The changes are accompanied by a loss of plasma volume (4—16%) [3–9]. Erythrocyte and hemoglobin concentrations in the blood remain constant, suggesting that the changes are driven by a feed-back mechanism. Immunological changes consist mainly of reduced T-lymphocyte reactivity. The results of the 96-day and 140-day Salyut-6 missions suggest that the adaptation of the immune system to spaceflight occurs in two stages: The first takes

place during the first 2—3 months in space, the second follows and consists of further weakening of the immune response [9–14].

It is important to point out that the RBC mass reduction and the depression of lymphocyte reactivity never harmed the health of the crews. The changes reflect physiological adaptation reactions rather than pathological conditions. However, with the advent of the Space Shuttle and of large space stations, the opportunity of flying in space will be offered to a broader community. The selection of crews will be less severe than in the past and therefore the hazard of anemic diseases and infections will be higher. The causes of the changes are not fully understood and further investigations are needed to explain these phenomena.

In addition to the medical examinations routinely performed, it will be useful to investigate systematically in vitro some of the important cellular processes which appear to be influenced by the space environment. This kind of experiments, when performed on space laboratories, will permit to discriminate between the effect of stress of spaceflight on the organism and the effect of $0 \times g$ on the biological system under investigation. This will deliver an important contribution to applied and basic research in space. As shown in Sect. 5.1.1 certain aspects of the immune system can be investigated in vitro.

2.2 Cell Biology

One of the most appealing features of experiments with living cells in weightlessness or at high-g is the transformation of gravity, a physical entity always constant in our ground laboratories, into a variable parameter like temperature or concentration. Consequently, living organisms which underwent evolution and development in a constant gravitational environment are suddenly confronted with a new situation. Therefore, the survival and proliferation of mammalian cells in altered gravitational fields is a challenging aspect of space biology.

The effect of varying the gravitational fields (mainly high-g) has been investigated on a variety of living organisms as long ago as 1806 (see Ref. [15] for a summary). The studies included plants, frog and sea urchin eggs, bacteria and amoeba, as well as complex organisms such as rat and man. Generally, the effects are more dramatic with increasing complexity of the investigated organism. A more detailed description of experiments with isolated cells in space is given in Sect. 3. Calculations made by Pollard [16] show that the distribution of cell organelles like mitochondria, nucleus, nucleolus and ribosomes may be influenced by gravity provided there is sufficient freedom of movement within the cell. This condition is satisfied in cells larger than 1 μm. Therefore, animal cells should be more subjected to gravitational effects than bacteria. In fact, when bacteria were grown in a centrifuge at $50.000 \times g$ [16] no effect was observed. However, the calculations of Pollard do not take into account cytoplasmic interactions like those involving cytoskeleton. In fact, intracellular movements are severely impaired by rather rigid structures. Gravity may interfere with cytoplasmic streaming as calculated by Kessler [17]. Folkman and Moscona [18] described the correlation between cell shape and growth: Cells of various lines in suspension are spherical whereas cells adhering to the walls of a culture flask are rather flat. It was found that when cells are converted from a flat shape into a spheroidal shape, cells incorporate less ^{3}H-thymidine into DNA. These findings provide further

arguments in favor of experiments studying the effect of gravity on cell proliferation since one can expect that cell shape is influenced somehow by forces and tensions related to gravity.

However, Todd [19] detected no influence of gravity on the behaviour of the mitotic spindle in cultured human kidney cells. No effect of spaceflight was observed on human embryonic cells flown on Skylab 3 [15]. However, none of the experiments mentioned involved cell differentiation events.

Our findings with lymphocytes cultured at different g-levels are described in Sect. 8. Briefly, high gravity has a stimulatory, simulated low gravity an inhibitory effect on lymphocyte activation by mitogens.

2.3 Radiobiology

Several kinds of radiations were detected in space ranging from UV and X-rays to high-energy particles. Among the high-energy particles (electrons, protons and heavy ions) the most important for radiobiologists are the HZE (high-charge and high-energy) particles, mainly iron nuclei. Although astronauts were hit by HZE particles, registered as flashes of light, during the past missions, no consequences for their health has been reported. Calculations for a standard Space Shuttle/Spacelab orbit show that flux of Fe nuclei is approx. 2.5 Fe cm^{-2} sr per day at solar maximum activity and 0.8 Fe cm^{-2} sr per day at solar minimum activity. About 10—20% of Fe ions have energies less than 500 MeV/nucleon, and are highly ionizing. About 25% of Fe nuclei will interact with an aluminium wall of 5 g cm^{-2} thickness. This is the minimum wall thickness encountered by a particle penetrating into the Spacelab.

Particular caution should be taken in case of solar flares: the radiation consists of 95% protons. Surface doses may be higher than 1000 rads; however, the penetration of the radiation is quite low. The effect of cosmic radiation on biological objects was studied on Biosatellite II, on Apollo 16/17 and on the ASTP missions (see Sect. 3.1). Cell damage like cell death, tumor induction and genetic mutations may occur at different levels, depending on the organelles hit.

Within certain limits, the study of cosmic radiation effects can also be performed on the ground with the accelerators now available. However, a combined effect of radiation and microgravity can be achieved only in space laboratories. Finally, every biological experiment in space should take into account the effect of radiation. Dosimeters will record radiation in different locations on Spacelab.

2.4 Exobiology

The objectives of this discipline are to study origin of life in the universe and the detection of extraterrestrial life. Simulation experiments with primordial elements like hydrogen, oxygen and nitrogen showed that simple molecules (methane, water, ammonia) can be formed in a primitive atmosphere. These molecules can react with one another and produce amino acids, purines, pyrimidines and carbohydrates, which are the essential constituents of the molecules of life. The first and only attempt to search for extraterrestrial life through a biological approach was performed on the Viking missions in March 1975 (see Sect. 3.4). It may be of interest to the reader to know that the total number of technological civilisations which have appeared over the entire history of our galaxy has been estimated to be around a billion.

Table 1. US Missions carrying biological payloads

Mission	Date	Duration	Biological specimens	Hardware	Comments
Discoverer XVII	1960	3 d	Human γ-globulins, rabbit antiserum	Millipore filters, nuclear emulsion, tracking paper	Greater Ag/Ab reactivity
			Human conjuctival and synovial cells	Culture chambers, refrigeration units, dosimeter	Failed: Medium exhausted
			Chlostridium sporogenes, *Chlorella ellipsoidea*	Glass ampoules	No effect of spaceflight
Discoverer XVIII	1960	3 d	Human amnion, conjunctival, sternal marrow, synovial monocytic, leukemia, HeLa, embryonic lung, and chicken embryonic cells	Glass ampoules, refrigeration unit, filmpack	Failed due to unfavorable experimental conditions
			Clostridium sporogenes	Glass ampoules	No effect of spaceflight
			Neurospora crassa	Millipore filters, photoemulsion	No effect of spaceflight
Discoverer XXXII	1960	3 d	Corn seeds (*Zea mays*)	Container	No effect of spaceflight
Mercury 5	1961	34 h	Chimpanzee	Life supporting system, sensors, photographic equipment	No effect of spaceflight, animal survived without damage
Gemini 3	1965	5 h	Whole human blood	Aluminium holder, ^{32}P, dosimeter	No effect of spaceflight
			Sea urchin eggs	Container with fixative operated through-handle	Failed for mechanical reasons
Gemini 8	1966	11 h	Frog eggs	Chamber with holder for chemical fixation	No effect of spaceflight
Gemini 11	1966	3 d	*Neurospora crassa*, whole human blood	Aluminium holder, ^{32}P, dosimeter	No effect of spaceflight
Gemini 12	1966	4 d	Frog eggs	Same experiment as on Gemini 8	
Biosatellite I	1966	—	Several biological experiments		Spacecraft did not react on command and burned on reentry after 60 d on orbit
Biosatellite II	1967	2 d			Part of the scientific yield reduced by the early reentry of the spacecraft

			Amoeba, *Artemia salina*, wheat seedlings, *Saccharomyces cerevisiae*, *Neurospora crassa*, *E. coli*	Package, thermistors, dosimeters, cytospectrophotometer, photocameras	No effect of spaceflight
			Wasps		Change in behavior
			Pepper plant		Greater amino acid changes, response to 0 × g similar to that in clinostat
			Tradescantia		Disturbed spindle function of root tips
			Salmonella typhimurium		Faster growth
			Frog eggs	Acrylic modules, thermistors	No effect of spaceflight
			Drosophila	Package, ^{85}Sr	Inconclusive results due to contamination of capsule with chemicals. 0 × g and radiation cause premature aging and chromosome damage
			Tribolium confusum	Housing compartment, ^{85}Sr, dosimeter	Abnormalities due either to 0 × g and radiation or to temperature drop during flight operations
Biosatellite III	1969	8.5 d	Pig-tailed monkey	Life-supporting system, several sensors	Early call down due to deterioration of physiological conditions. Death 8 h after recovery due to dehydration and electrolytic imbalance
OFO-1(A)	1970	6 d	Bull frog	Animal immersed in water, sensors, life-supporting system	Only partial adaptation of the behavior to 0 × g
Apollo 14	1971	9 d	DNA, hemoglobin, dyes	Zonal electrophoresis equipment	Feasibility of electrophoresis at 0 × g demonstrated by separation of dyes
Apollo 16	1972	11 d	Drosophila	Package, ^{85}Sr	No effect of spaceflight
			Artemia salina and grasshoppers eggs, *Tribolium confusum*, *Arabidopsis thaliana* seeds, bean embryos, *Bacillus subtilis*, protozoan cyst	Biostack I	Discussed in detail

Table 1. (continued)

Mission	Date	Duration	Biological Specimens	Hardware	Comments
			Nematode larvae, *Bacillus thuringiensis*, *Bacillus subtilis* (4 strains), *E. coli* T-7 phage, *Chaetomium globosum*, *Trychophyton terrestre*, *Rhodotorula rubra*, *Saccharomyces cerevisiae*	Microbial ecology evaluation device with 798 cuvettes, dosimeter, thermometer	No effect of spaceflight
Apollo 17	1972	12 d	Biostack II: identical to biostack I		
			Pocket mice	Cannister, life-supporting system, dosimeters implanted in brain	Four mice of five returned alive, 80 HZE events recorded, no significant lesion found
Skylab 2	1973	28 d	*Bacillus subtilis*, *Bacillus mycoides*, *E. coli*	Petri dishes, incubator, photocamera	Faster growth, fewer but larger colonies, morphological changes, more sensitive to antibiotics
Skylab 3	1973	59 d	Antigens/antibodies	Immunodiffusion plates, thermos	No effect on Ag/Ab reaction
			Human embryonic lung cells	Incubator	Described in detail, no effect of spaceflight
			Elodea (water weed)	Vials, microscope, picture camera	Failed, plants died
			Rice seedlings	Package, camera	Irregular growth
			Cross spiders	Cage, photoequipment	Spiders use gravity-sensitive organ for web formation
			Killifish	Polyethylene bags, photoequipment	$0 \times g$ acts as vestibular stimulus: swimming anomaly
Skylab 4	1973/74	84 d	*Elodea*, *Bacillus subtilis*, *Bacillus mycoides*, *E. coli*	Petri dishes, incubator, photocamera	Elodea died, the other microorganisms showed the same changes as on Skylab 2
ASTP (Apollo-Soyuz Test Project)	1975	9 d	Human, horse, rabbit RBC, human lymphocytes and kidney cells, rat bone marrow, spleen and lymphnode cells	Zone and free-flow electrophoresis, isotachophoresis equipment	Separation and concentration of cells achieved, discussed in detail
			Streptomyces levoris	Petri dishes, incubator, photocamera	Decreased growth rate periodicity, no changes in biorythm

		Artemia salina, *Tribolium confusum*, *Carausius morosus*, tobacco seeds, *Arabidopsis thaliana*, *Zea mays*, *Bacillus subtilis*	Biostack III	Discussed in detail
		Killifish embryos	Polyethylene bags, photoequipment	No effect on embryonic development
Viking	1976	Mars soil	Fully automated equipment for search of primitive life on Mars	Discussed in detail

3 Biological Payloads on US Missions

In this section experiments performed aboard manned and unmanned US space stations are reviewed. As pointed out before, very little research has been done aboard manned satellites. A number of interesting experiments were carried out on automated satellites, mainly on the US Biosatellite II. However, some projects failed for mechanical reasons.

We discuss here in detail those projects which are most relevant to space biotechnology. A concise summary of all biological experiments carried out on US missions has been recently published by NASA [20].

A consistent program of basic and applied research in life science will start when the Spacelab becomes operational. There, facilities like incubators, centrifuges, microscopes, freezers and refrigerators will offer to the user acceptable working conditions (see Sect. 5). In addition, a selected group of scientists is presently trained by NASA as mission and payload specialists for biological experiments. However, we must always keep in mind that safety requirements and constraints like weight, energy and crew time will always limit the scientific goals of the investigations.

Two aspects are relevant: a) The instrumentation carried by the space vessel, and b) the effects of the space environment on growth and behaviour of the organism studied. Table 1 compiles the biological experiments carried on US missions so far [20]. Biomedical investigations on humans or experiments with animals or plants are not discussed. The instrumentation employed was, except for few cases, very simple and mainly consisted of passive containers (petri dishes or sealed ampoules) in which the microorganisms were kept in a nutrient medium. The temperature control was rather primitive and mainly consisted of thermos containers. Man power invested in biological experiments was very limited or nil. The duration of the missions was in most instances too short for an extensive study of the effect of spaceflight on growth and survival of microorganisms. The interest of the investigators was directed toward the detection of radiation damages rather than toward the effect of weightlessness per se. Two of the most important missions, namely Biosatellite I and II either failed or landed too early, upsetting the schedule of most experiments. Therefore, it would be premature to draw conclusions on the adaptation of living systems in space from the studies which have hitherto been performed. We should use the experiences gained for a better choice of the system to investigate and for improving the reliability of the equipment. More biochemical parameters should be analyzed in the future. DNA, RNA and protein biosynthesis are useful indicators of intracellular changes still unexplored in weightlessness. Table 1 shows that very little has been done in this direction particulary with animal cells. In addition, differentiation processes have never been studied in vitro. Lymphocytes and hemopoietic cells are good test objects for this purpose (Sect. 5.1.1). Four relevant projects performed on US missions are discussed here in more detail.

3.1 Biostack Experiments

Biostack was one of the first sophisticated experimental devices carried out in space. It consisted of a passive container which did not require power or crew interface. Biostacks I, II and III were flown on the Apollo 16, 17 and Apollo-Soyuz Test Project

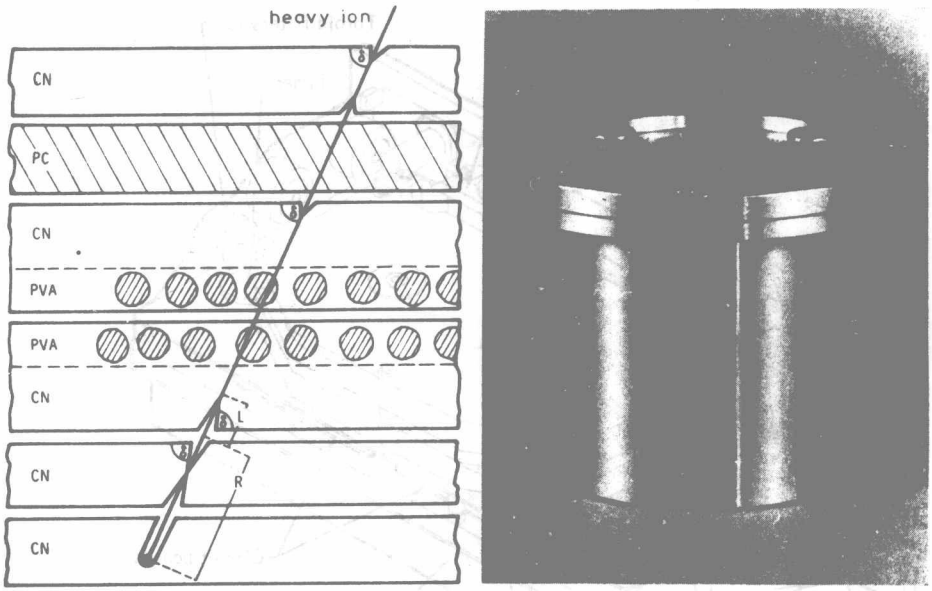

Fig. 1. Schematic representation of the biostack conception. Left: Monolayers of biological objects are sandwiched alternately with nuclear track detector sheets. Right: Biostack flight container. CN: cellulose nitrate, PC: polycarbonate, PVA: poly-(vinyl alcohol) (courtesy of H. Bücker, DFVLR, Frankfurt)

(ASTP) missions, respectively. The biostack experiments were designed and evaluated by a group of 30—40 investigators in Europe and USA [20]. The objective of these experiments was to study the effect of cosmic radiation on a variety of biological objects (Table 1): Microorganisms, eggs and plants seeds were embedded in poly(vinyl alcohols) between photographic emulsion layers (Fig. 1). This concept first proposed by Eugster [21] and developed by Bücker [22] allows the identification of the object hit by the radiation and to track the path of the penetrating particle through the biological object.

Eggs and plant seeds were grown after landing in the ground laboratories. Several effects of radiation were detected. Most interesting are *Artemia salina* eggs developed to individuums with abnormalities in the extremities, in the abdomen and in the thorax. *Zea mays* seeds produced plants with leaves showing large unusual yellow strips.

Biostack is a typical example of a simple, passive equipment without energy or crew operation requirements with a high scientific yield. However, the equipment is suitable only for investigations of the effect of radiation whereas gravitational effects cannot be detected with this approach.

3.2 Tissue Culture Incubator on Skylab

The effect of spaceflight on human embryonic lung cells WI-38 was studied during the 56-day Skylab 3 mission by Montgomery et al. [15, 23]. The experiment was

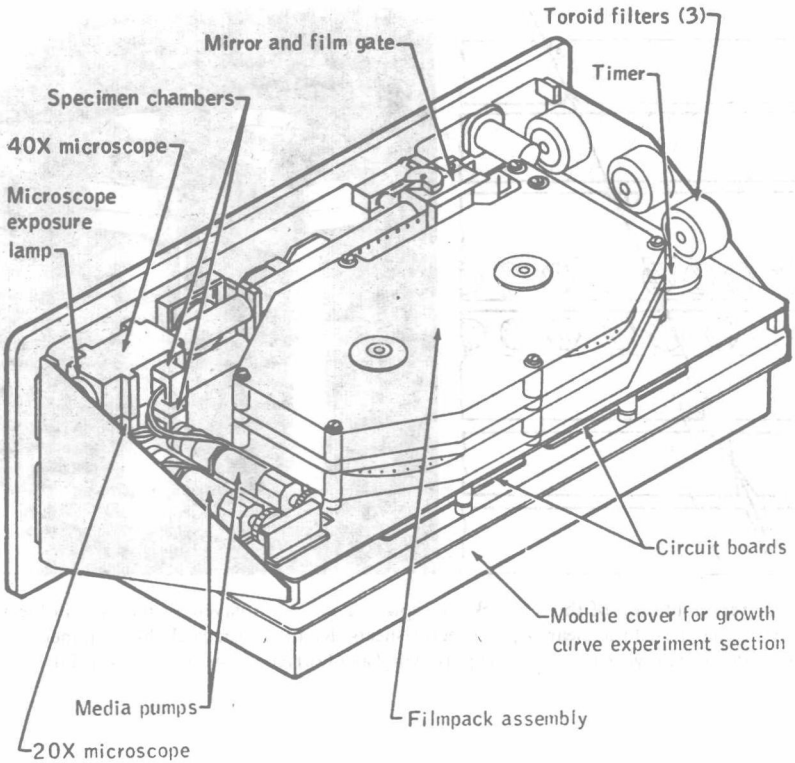

Fig. 2. Internal set-up of Woodlawn Wanderer 9 incubator (courtesy of R. G. Thirolf)

performed with one of the most sophisticated equipment ever used in space biology[24]. It consisted of a fully automated tissue culture package called Woodlawn Wanderer 9, designed to achieve four major objectives:

— To keep cells alive in culture by supplying fresh medium at 36 °C for several days;
— to record phase contrasted pictures with a time-lapse camera for 28 days;
— to fix several specimens of cells at given times;
— to recover living cells after flight for further culturing in the ground laboratory.

The instrument weighed 10 kg, measured 40 × 19 × 17 cm and was powered by 28 DC with an average consumption of 16 W at 10 °C room temperature. The package was separated into two main compartments: A camera-microscope section and a redundantly sealed growth-curve experiment section (Fig. 2).

The camera-microscope section consisted of two independent 20 and 40-power *camera microscopes*. The pictures were recorded on a 16 mm microfilm. The cells were grown on glass in perfusion chambers filled with cultures containing 7000 cells/ml. After the cells became attached to the lower glass disk, the chamber was fixed in the microscope and focused. Each chamber had a volume of 105 µl and was connected to an automatic perfusion system adding fresh medium every 12 hours.

The growth-curve experiment section was contained in a module easily removable for biological servicing and consisted of two separated identical and independent