SESSION II

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ZERO-GRAVITY TISSUE-CULTURE LABORATORY

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ABSTRACT

Hardware has been developed for performing experiments to detect the effects that zero gravity may have on living human cells. The hardware is composed of a time-lapse camera that photographs the activity of cell specimens and an experiment module in which a variety of living-cell experiments can be performed using interchangeable modules. The experiment is scheduled for the first manned Skylab mission.

INTRODUCTION

It is possible that the removal of gravity for an extended period of time will have significant effects on living organisms. These effects may be manifested at the cellular level because this is the fundamental biological element in all living systems.

Living cells are studied routinely outside the living system by supplying them with oxygen and suitable nutrients, by maintaining their temperature, and by protecting them from toxic and disease-causing agents. This process is known as tissue culture. Typical experiments include microscopic observation of the tissue-culture cells, measurement of rates of nutrient usage, and observation of the effects of chemical, radiation, disease-inducing agents, and many others.

A small, automated tissue-culture laboratory has been developed for the National Aeronautics and Space Administration (NASA) that will be used to perform a variety of experiments designed to determine the effects of the zero-g environment on living cells.

The laboratory was designed to achieve two major objectives: to produce time-lapse movies of living cells and to perform a variety of cell experiments by injecting various liquids into the nutrient media. All data are to be analyzed after recovery of the flight package.

As with most equipment developed for space flight, the laboratory-hardware design is restricted by the usual limitations of size, weight, and power; at the same time, high reliability and safety standards must be maintained. In addition, the biological compatability of materials and the necessity to sterilize components at a high temperature are major constraints.

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We wish to thank Harry Jarrett and Richard Summers for contributions to the design of the equipment.

DESCRIPTION

The laboratory is enclosed in an airtight package that weighs 11 kilograms and occupies 0.015 m^3. Switches on the faceplate of the package (fig. 1) are operated by the crewmen in flight to initiate operations and, with the aid of indicator lamps, to check equipment performance.

Internally, the package is divided into two operationally independent sections to accomplish the two major objectives. The two divisions are the camera-microscope section and the experiment module. The camera-microscope section and the relative location of the experiment module are illustrated in figure 2. Space between the two sections is provided for the addition of circuit boards and timers. A container slips over the entire assembly and joins with the front panel to form an airtight enclosure. All penetrations of the panel by switch shafts and windows are sealed with O-rings.

Camera Microscope

The camera-microscope section consists of two independent systems. One system photographs specimens through a 20-power microscope and the other system photographs through a 40-power microscope. Each microscope, including the lamp used to illuminate the specimen, is only 7 by 4 by 2.5 centimeters in size. The phase-contrast image produced by the microscope is projected through a tube and is reflected by a mirror onto the film. The mirror can be rotated, permitting an observer to view the image and to focus the microscope during ground-based checkout. A locking device is provided to hold the objective lens in position after focusing. No shutter is required because the lamp is turned off after each exposure.

The camera operation (fig. 3) is unusual in that film registration is accomplished by using photocells to sense sprocket-hole position in the film instead of using the usual sprocket wheels or claws. This arrangement reduces weight and eliminates troublesome film loops and takeup-reel slip clutches. It is limited in speed, however, because it requires the takeup reel to stop for each frame. Periodically, an internal timer turns on the microscope exposure lamp for a short period of time to expose one film frame. As the lamp turns off, a pulse is generated that triggers a silicon controlled rectifier (SCR). The SCR completes a circuit that allows the film-takeup drive motor and registration lamp to operate. The motor advances the film until a small light beam shines through the next sprocket hole and strikes the phototransistor. The phototransistor output switches on a transistor connected across the SCR. The SCR stops conducting because of the shunt path, but the transistor maintains the circuit until the edge of the sprocket hole again interrupts the light path, turning off the transistor and stopping the motor. Now, the cycle is ready to be repeated for the next frame.

Film for the camera is supplied from a replaceable film pack that has two rolls of 16-millimeter film that is 100 meters long. Normally, the photographic rate is 5 frames/min for 40-minute intervals twice each day throughout the 28-day mission.

Each microscope holds a living-cell specimen grown in a 0.05-cm^3 chamber formed by a gasket sandwiched between two glass disks. Tubes are attached to the gasket for use in injecting fresh liquid nutrient media and for removing waste media. The chamber is kept in a heated block that is thermostatically controlled to maintain body temperature (310.15° K or 37° C).

Cells suspended in a liquid medium are injected into the chamber. After a few hours, the cells settle into a thin layer and firmly attach to the lower glass disk. Then, the chamber may be installed in a microscope in any position. The thin layer of cells forms a flat plane on which the objective lens is focused.

Fresh nutrients are supplied twice a day from a cylindrical reservoir containing a piston. The piston forces fresh media into the chamber and forces waste media into the volume on the back side of the piston. The piston is advanced by a motor that rotates a lead screw on which the threaded piston rides. Separate media-pump reservoirs are provided for each specimen chamber. Timing of the camera cycles and the media-pump advance is controlled by a commercially available, self-powered, tuning-fork watch movement that operates switch contacts.

Experiment Module

This module section is constructed so that various hardware modules can be installed in the basic package to perform a variety of experiments. The module is enclosed in a sealed container, which provides redundancy in case the outer enclosure seal is lost. Two different modules have been designed. One module, termed a biopack, has been designed to inject various radioactive tracers (or labels) into specimen chambers after several days of cell growth in a zero-g environment. Then, the labels are rinsed out and the cells are preserved with a fixative solution for analysis after recovery of the equipment. Analysis includes the measurement of the incorporation of the radioactive compounds by the cells.

The cell-growth curve module is designed to grow cells in several chambers. At predetermined intervals, the chambers automatically fill, one at a time, with a fixative that is used to preserve the cells. Upon return of the package, a growth curve will be constructed by counting the cells in each chamber and tabulating the number according to the day they were preserved. Any shifts in this curve compared with ground-based control experiments may be a result of the zero-g environment.

Biopack Module

The biopack module is installed in the package with one experiment assembly removed (fig. 4). The module consists of two experiment assemblies, one of which labels, rinses, and fixes the cells on the fourth day after launch. The other module labels the cells on the 10th day after launch. The label/rinse/fix cycles are initiated by the astronauts. The experiment assemblies are identical except that the 10-day media-supply pump is longer. Both assemblies can be removed from the drive-train portion of the module for sterilizing and filling with biological materials.

Each experiment assembly has 12 chambers that are used for maintaining living cells. The chambers are connected in series so that, for feeding twice a day, fresh media are forced into chamber 1 and the contents of chamber 1 are forced into chamber 2 and so on through chamber 12. Enough media are provided from a media-pump reservoir to change the media in all 12 chambers each time the cells are fed. This feeding process is performed automatically until either 4 or 10 days after launch. On these days, an astronaut will operate a switch to begin the label/rinse/fix cycle of the appropriate biopack.

In order to label, rinse, and fix the cells, the equipment must perform the following operations in sequence.

1. Shift a valve in the chamber block to disconnect the series fluid path and to connect individual chambers to separate pump reservoirs containing label and rinse solutions.

2. Inject the various radioactive labels into the proper chambers.

3. Rinse out the labels after a predetermined time.

4. Return the chamber valves to the series feed position.

5. Inject the fixative into all chambers.

6. Shut off power to the experiment assembly.

The label and rinse pump reservoirs are constructed from a solid block containing 11 bores (one of the 12 chambers is not labeled). A piston and a lead screw are installed in every bore. Initially, each piston is positioned so that the reservoir volume on one side holds 2 cm^3 of a label solution. Rinse solution fills the 7-cm^3 volume on the other side. Tubing connects the two ends of each pump to the proper chamber. As the piston is advanced, the label solution is forced into the chamber and the chamber contents are forced into the rinse-solution side of the pump. Reversing the piston forces the rinse through the chamber into the end of the pump that originally contained the label.

All of the various pumps and valves are driven by means of a single drive motor (fig. 5). In order to select which device is driven at a given time, an escapement mechanism is used. The motor rotates a shaft that would be free to slide along its axis if it were not restrained by the escapement. A spring on the shaft keeps the escapement pressed against a stationary pin. Initially, a gear attached to the shaft is alined to drive the media pump. Twice each day, the internal timer starts a media-advance cycle. This cycle is terminated by a cam switch after the proper number of revolutions. When the astronaut is ready to begin the label/rinse/fix cycle, he rotates a front-panel switch causing the motor to run in reverse. This reversal rotates the escapement enough to allow it to slip a short distance past the stationary pin. The spring forces the shaft and drive gear forward to engage the gear that drives the chamber-valve shifting mechanism. The motor continues to drive until a limit switch terminates the cycle. The motor direction is changed for each succeeding step to engage the proper gear at the right time.

When the drive gear shifts, it may not engage properly at first, but a few degrees of rotation will allow the gears to mesh. This drive normally operates only a few times during testing and flight and the motor speed is only 20 revolutions/min. Test units have been subjected to hundreds of operations without any significant wear. This system is smaller, lighter, and less complex than a comparable system that has electromechanical clutches or numerous motors.

A pump similar to the media pump (but smaller) is used to supply the fixative solution. The fixative is introduced through the same series path that the media flowed through. A selector valve is repositioned when the chamber valves are first shifted, connecting the fixative pump to the fluid lines and disconnecting the media pump.

Growth-Curve Module

The growth-curve module consists of two identical, independent assemblies. Each assembly provides nine cell-culture chambers installed in a temperature-controlled holder (fig. 6). The specimens are fed automatically by means of a media pump. Media pass through the preheater (a reservoir attached to the heated holder) before entering the chambers.

A tape program reader, driven by the media-pump motor, uses a Mylar tape that is punched with two rows of holes. Microswitch actuators ride on the tape and drop into the holes. One row of holes determines the amount of media for each feeding, and the other row initiates cycles to inject fixative into one chamber at a time. Usually, fixation will be programed to occur at 2-day intervals.

Fixation is accomplished in the following manner. Upon command by the programer, the fixative-pump motor begins to drive a sector gear. The sector gear rotates a fixing valve 22.5°, then disengages. This rotation connects lines from a fixative pump to the chamber to be fixed and disconnects the chamber from the media-supply lines. As the motor continues to run, the sector gear engages a gear on the fixative pump. Fixative is injected and the cycle is terminated by a cam switch on the motor. This cycle is repeated to fix the other chambers. One of the nine chambers is not fixed but is returned with the cells alive so that they can be subcultured upon return to earth.

Each time a specimen is fixed, it no longer requires feeding. The tape programer terminates the media-pump cycle sooner so that only media enough for the remaining unfixed chambers will be supplied.

DESIGN PROBLEMS

When living cells are maintained and studied in test tubes, bottles, and so forth, they are said to be studied "in vitro." The term literally means "in glass." Most tissue culture is still performed "in glass" because most glass is nontoxic to the cells. A few other materials, such as Teflon and silicone rubber, are used routinely. The development of the flight equipment required considerable research to find materials that have properties of nontoxicity, structural strength and stability, machinability, heat resistance (for sterilizing at 393.15° K or 120° C), and flame resistance (for use

in manned spacecraft). Clear sulfuric-acid-anodized aluminum (type 6061) is used extensively because it has all of the properties just noted and it is light in weight. Care must be exercised to prevent scratching the surfaces, however, because unanodized aluminum is toxic. The toxicity of each lot of material must be tested before use because minor changes in its manufacture can make it unusable. For example, "nontoxic" epoxy can be lethal to cells if curing-agent proportions are varied.

Another problem encountered when performing tissue culture in zero-g is bubbles in the fluids. Normally, gravity holds fluids in contact with cells, and bubbles are of little consequence. Because in zero-g the location of bubbles cannot be predicted, it is necessary to build fluid systems so that bubbles may be removed during filling operations. The use of materials, such as silicone rubber, that have a high permeability to gases is restricted because gas leakage will cause bubbles to form after a period of time.

CONCLUDING REMARKS

Many new techniques for performing tissue culture have been devised in the development of zero-g tissue-culture equipment. Several of the techniques can be adapted to laboratories on earth. These techniques would be of particular value in long-term experiments to eliminate daily manual care and, by using closed systems, to reduce the risk of contamination.

DISCUSSION

K. M. Speight:

What type of motor is used in the main drive?

Cook:

Direct-current brush-type generators are used. Because our package is sealed, we do not operate in the pure-oxygen atmosphere of the command module.

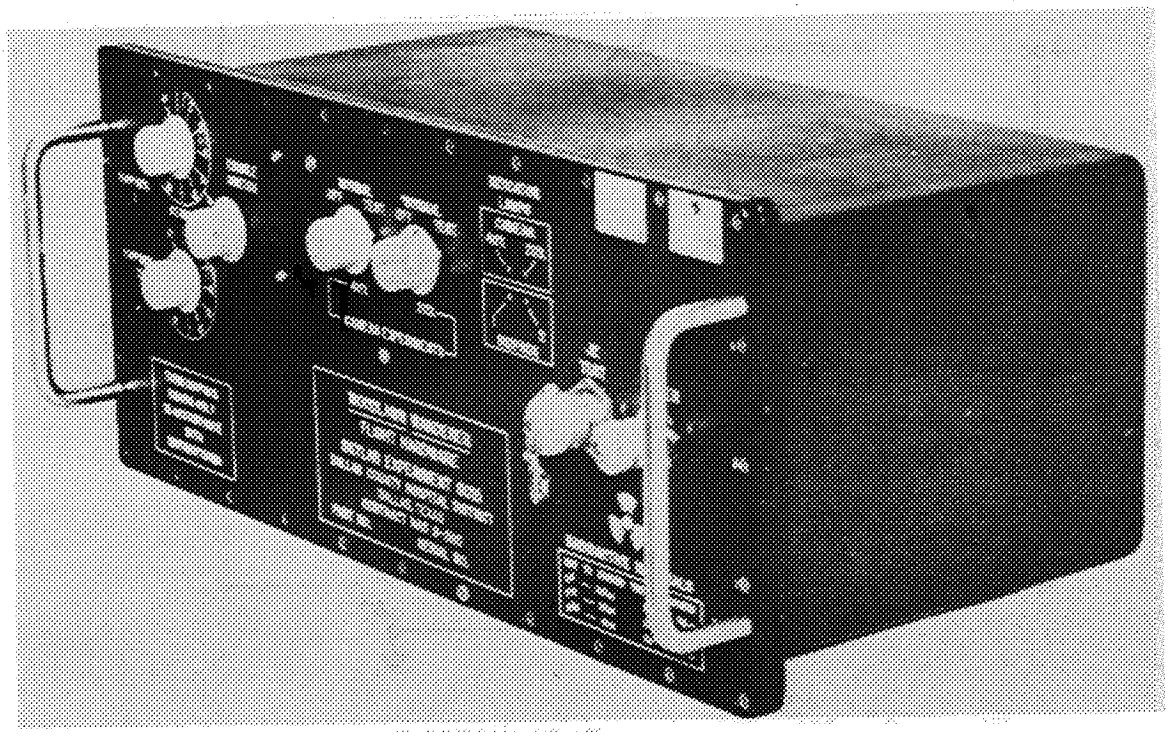

Figure 1. - Flight-hardware package.

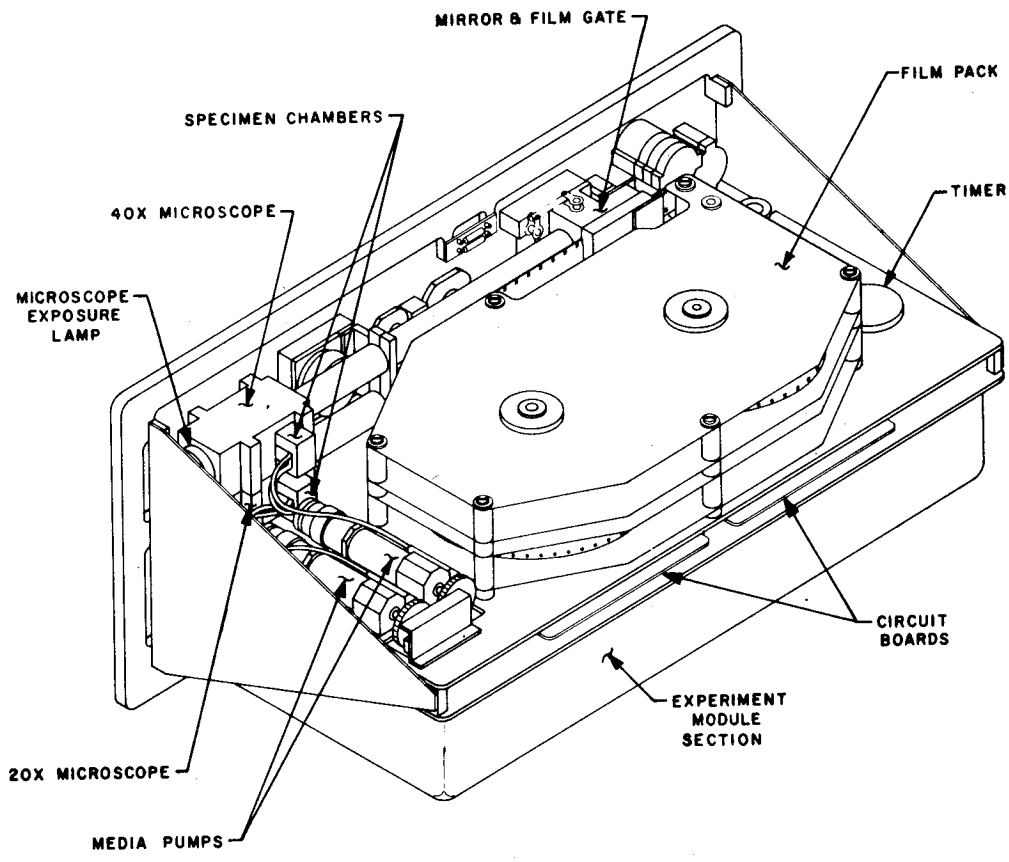

Figure 2. - Camera-microscope section.

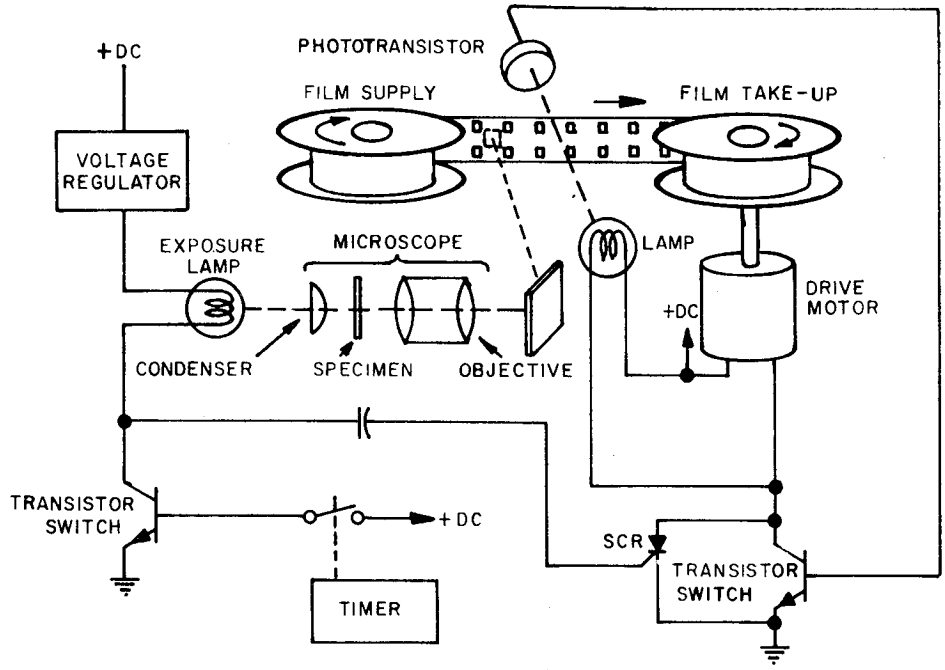

Figure 3. - Microscope-camera diagram.

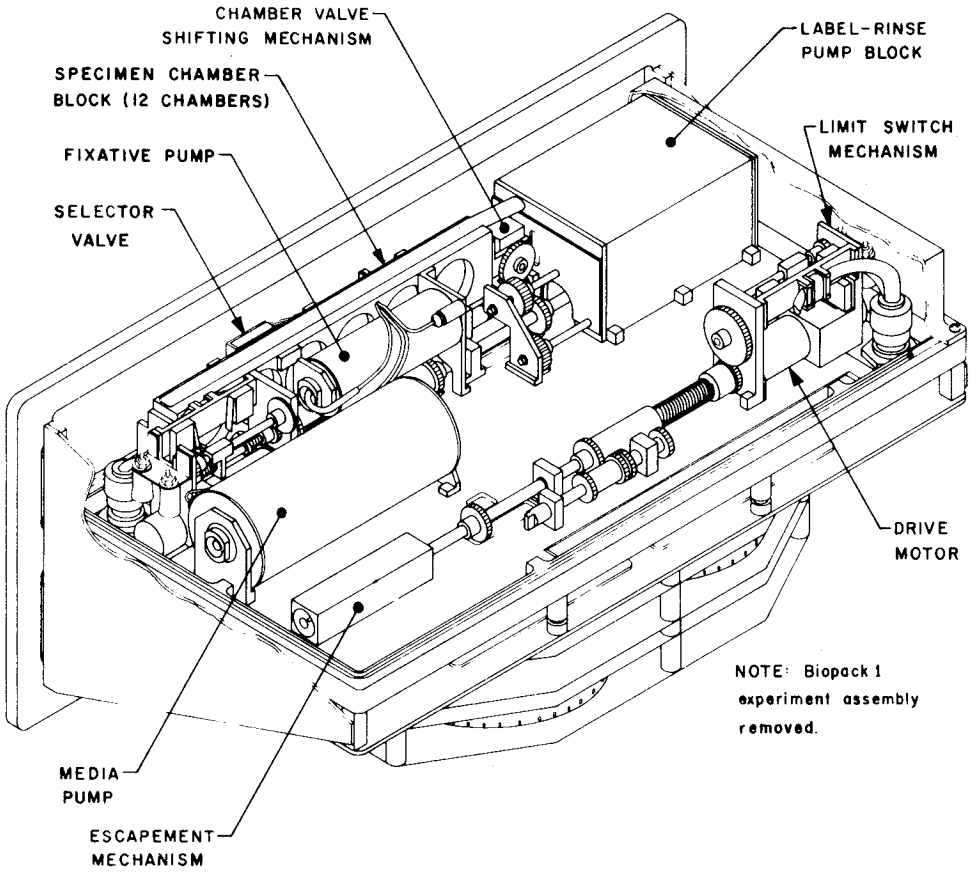

Figure 4. - Biopack experiment module.

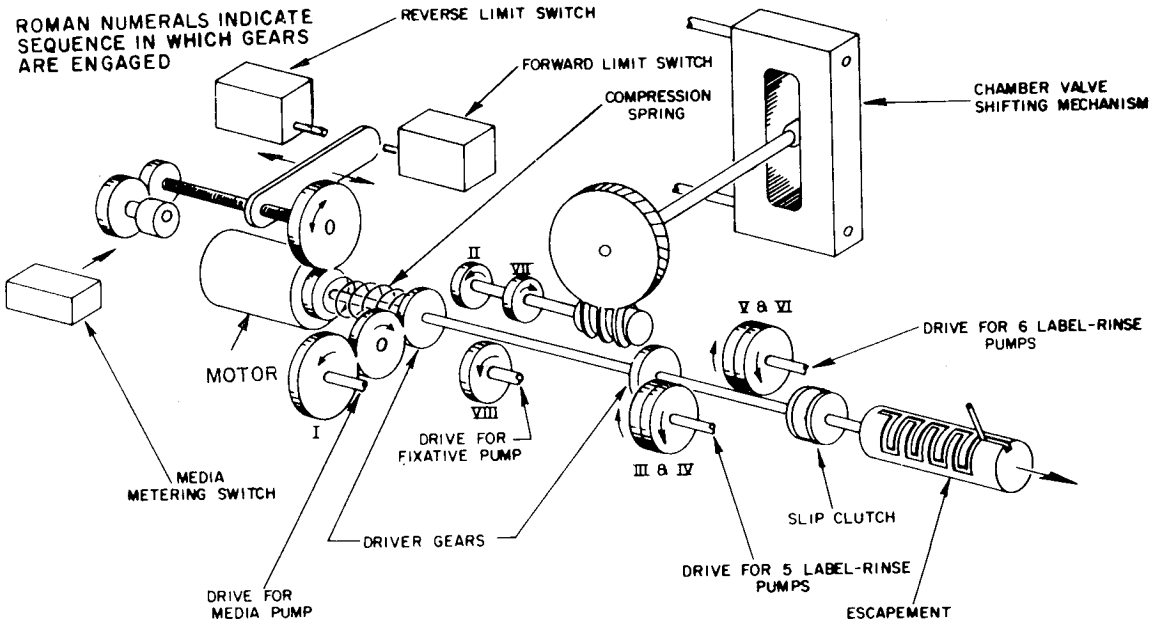

Figure 5. - Biopack drive train.

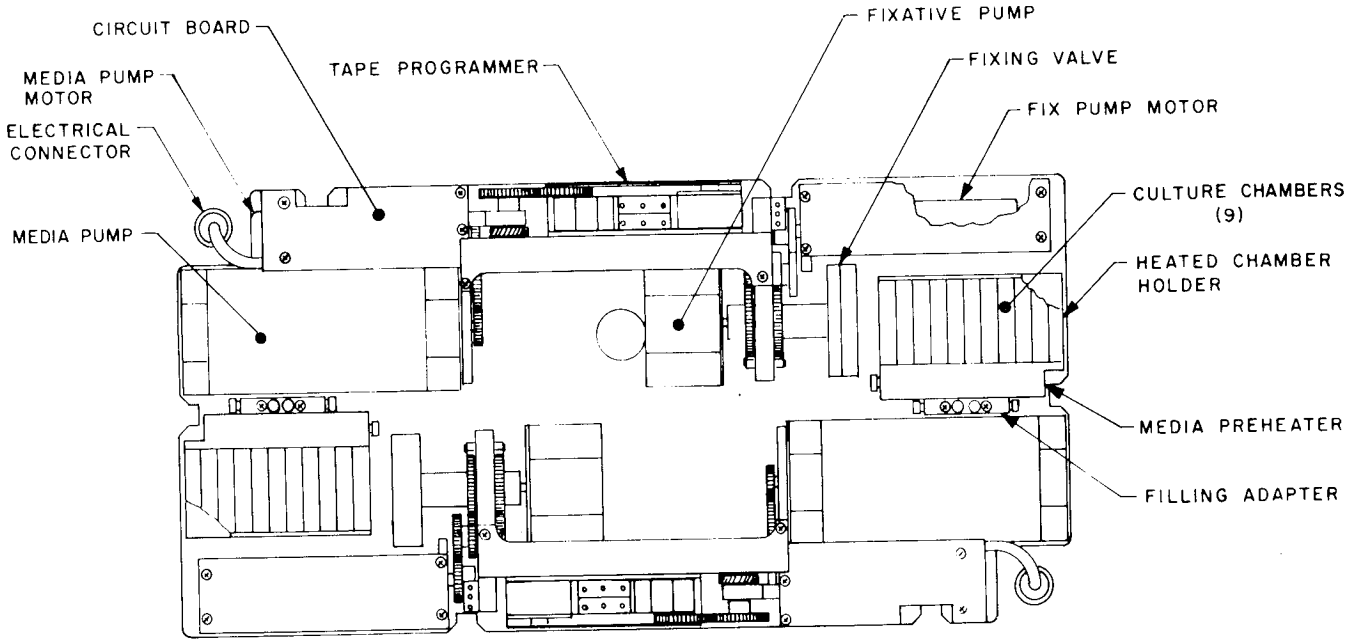

Figure 6. - Growth-curve module.