CELLULAR EFFECTS OF MICROGRAVITY

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In the 1960's Soviet and U.S. investigators studied a variety of biological specimens on unmanned space vehicles. Most of these simple experiments reported normal growth and function, however, a few isolated results indicated increased growth in procaryotic cells (Taylor, 1974-review). Experiments with E. coli K-12 (lambda) phage on over 20 Soviet flights, beginning with Vostok series, showed an increased phage induction in lysogenic bacteria, which was correlated with mission duration (Parfenov and Lukin, 1973). The role of microgravity, however, was partially obscured by contribution of galactic radiation and some evidence that launch vibrations followed by increased radiation may increase the mutation rate (Zhukov-Verezhnikov et al., 1965). Flight experiments on Biosatellite II indicated that the cell density of Salmonella typhimurium cultures were greater than control cultures on Earth (Mattoni, 1968). During the 1970's a number of microbial studies were conducted to sort out the relative effects of microgravity and increased radiation exposure during orbital flight (Taylor,1977). Since then only a few cell biology experiments with insect, mammalian and plant cells have been flown (Taylor,1986). Figure 1 summarizes the significant results (excluding plant cell effects) indicating direct effects of microgravity on cells in vitro.

Only a few experiments have been performed on eucaryotic cells. These were confined to small holding chambers maintained at constant temperatures permitting only basic observations on orbit and occasional sample fixation for post flight analyses. No experiments have yet been possible in a carefully controlled perfusion bioreactor wherein nutrients can be constantly replenished and waste

products removed to avoid toxic levels.

Although Skylab experiments with WI-38 lung fibroblasts indicated that subcellular structure and growth was unchanged some indication of increased glucose utilization was observed(Montgomery, 1974). The noteable lack of major effects and theoretical predictions that subcellular organelles would not be subject to sedimentation or other gravitational effects resulted in dramatic narrowing of focus for U.S. cell biology flight experiments. From 1975 to 1981 current theories held that cell function and intracellular physiology would be unaffected by exposure to microgravity.

Recent findings, however, clearly demonstrate that cell functions are significantly altered by micro-G. French experiments on Salyut-6 & 7 with Paramecium tetraurelia showed clearly an increase in cell proliferation rates and cell volume after just 60 hours of spaceflight (Planel,1981 & Tixador,1984) Hungarian-Soviet experiments on human lymphocytes showed that in vitro cultures exposured to several interferon inducers on orbit caused the release of 4-8x more alpha-interferon than ground controls(Talas et al, 1983). In contrast lymphocytes from cosmonauts do not respond to interferon inducers after space-flight as they did preflight. Also in vivo Gamma-interferon production in rats on Spacelab-3 was severely inhibited (Gould et al, 1985). Swiss experiments on Spacelab-1 showed that Concanavalin A mitogen stimulation of lymphocytes in vitro was reduced by 97% in microgravity (Cogoli et al, 1984) in contrast to a 30% increase in mitogen response at 10-g's (Tschopp and Cogoli,1983). Dramatic reduction in mitogen response was also confirmed on the D-1 Spacelab mission as described below. Observations from STS-8 and definitive results from Spacelab-3 showed that in vitro mammalian pituitary cells did not release normal amounts of growth hormone during flight, Moreover, the growth hormone secretions from pituitary cells taken from the flight animals and transplanted postflight into hypophysectomized animals did not stimulate growth of long bones as did control

cells (Hymer et al, 1985). In contrast prolactin secretions from those cells appeared unaltered by the spaceflight. In vitro experiments on STS-7 and STS-8 determined the efficiency of human kidney cells attachment to collagen coated microcarrier beads (Tschopp et al, 1984). It was expected that attachment would be less after mixing in micro-g since opportunities would be limited by random collisions. However, results showed a significant increase in attachment within the first three hours (Morrison and Lewis, 1986). Once cells were attached, both the flight cells and the ground control cultures appeared to grow at the same rate, at least for the first 25 hours.

The most dramatic results have just recently come from the Spacelab D-1 mission, wherein the Biorack experiments studied the effects of micro-G on the structure, proliferation, differentiation and functions of some nine different procaryotic and eucaryotic cells (See Figure 2). For the first time inflight experiments were performed at both micro-g and 1-G and the results were compared to ground controls. Figure 3 summarizes the results (Mesland et al., 1986) which indicate significant effects of spaceflight on cell growth, development and function. These include evidence for increased resistence of E. coli to an antibiotic and increased proliferation rate and greater cell mass of B. subtilis cultures. Also transfer of DNA during conjugation of bacterial cells was 2-3x greater in microgravity. Cell proliferation of Paramecium sp. was increased 4x, cell volume was increased and intracellular calcium was decreased which confirmed earlier results from experiments on Salyut flights. The in vitro mitogenic response of human lymphocytes to Con A showed a 90% decrease under microgravity conditions, whereas the inflight 1-G control cultures showed a 60% loss of response compared to ground controls.

In general, these experiments clearly showed significant alterations of cell proliferation, differentiation, and embryonic development (insect cells).

These results have already created a renewed interest in cellular effects of microgravity which may provide new insight into some human physiologic responses to prolonged spaceflight. They also indicate that space biotechnology process studies need to include effects of microgravity on the specimens to properly assess the potential advantages and trade-offs for a particular biological application.

Future flight experiments will require more sophisticated culture facilities. Experiments with fragile mammalian cells will require a capability for growth and maintenance of a large number of cells in a precision controlled culture environment. Figure 4 illustrates a typical growth curve for kidney cells grown on microcarrier beads. Attachment and proliferation requires a complex culture medium usually supplemented with serum. All sera contain a variety of known and unknown "growth factors" in addition to a large complement of different serum proteins and hormones (Taub, 1982), some of which are inhibitors of cell secretions (Verheijen, 1986). During log growth nutrient consumption is varied and often rapid for certain critical molecules. Waste product accumulation also can be quite rapid. Often cell secretion of important hormones and other proteins is depressed or absent during this phase. Once adequate packing density or confluence is reached growth and metabolic rates become slower. Then culture medium can usually be switched to a more simple, defined formula which can be designed to encourage cells secretions into the medium.

The indicators for improved cultures under better controlled local environmental conditions are different for growth phases and cells maintained at confluence. During proliferation the main indicators which can be quantitated are attachment, viability, and replication rate. However, in stable maintenance culture one can measure cell survival, cell turnover, respiration and metabolic rates, specific nutrient depletion rates, changes from aerobic to anaerobic metabolism, and rates of product secretion. The first microgravity experiments in a

suspension-type Space Bioreactor are likely to be designed for separate studies of cells cultured either under proliferation conditions or under maintenance conditions specific for confluent cells with target secretory functions. Later flight experiments can be accomodated which look at the entire sequence of cell culture in a continuous operation where medium changeout can be accomplished via a dedicated microprocessor control system.

Some initial flight experiments have been proposed and concepts for other bioprocess experiments have been defined (See Figure 4). Various versions of the first Space Bioreactor also can be used to maintain cells under strict environmental conditions to support some of the more elaborate cell biology flight experiments. Typical experiment requirements are already being incorporated into the basic design of the first flight prototype.

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CELLULAR EFFECTS OF MICROGRAVITY

MISSION	CELLS	RESULTS
BIOSATELLITE	Salmonella typhimurium	growth increased
Salyut-6	Proteus vulgaris	growth increased
Voskhod 3	E. coli lambda phage	induction increased (20 flights
Skylab-3	WI-38 lung fibroblasts	normal growth (glucose inc.)
Salyut-6,7	Paramecium tetraurelia	growth rate & size increased
Salyut-6	Lymphocytes (in vitro)	interferon production incr. 4X
STS-8, SL-3	Pituitary cells	growth hormone secretion decr.
		normal prolactin secretion
SPACELAB-1	Human lymphocytes (in vitro)	mitogenic response decreased 97%
STS-8	Embryonic kidney cells	attachment increased 40 - 80%
D-1 SPACELAB	procaryotic & eucaryotic cells (9 types)	changes in cell proliferation and cell differentiation

FIGURE 1

FIG. 2 - BIORACK EXPERIMENTS FOR THE D-1 MISSION

From Mesland et al , 1986
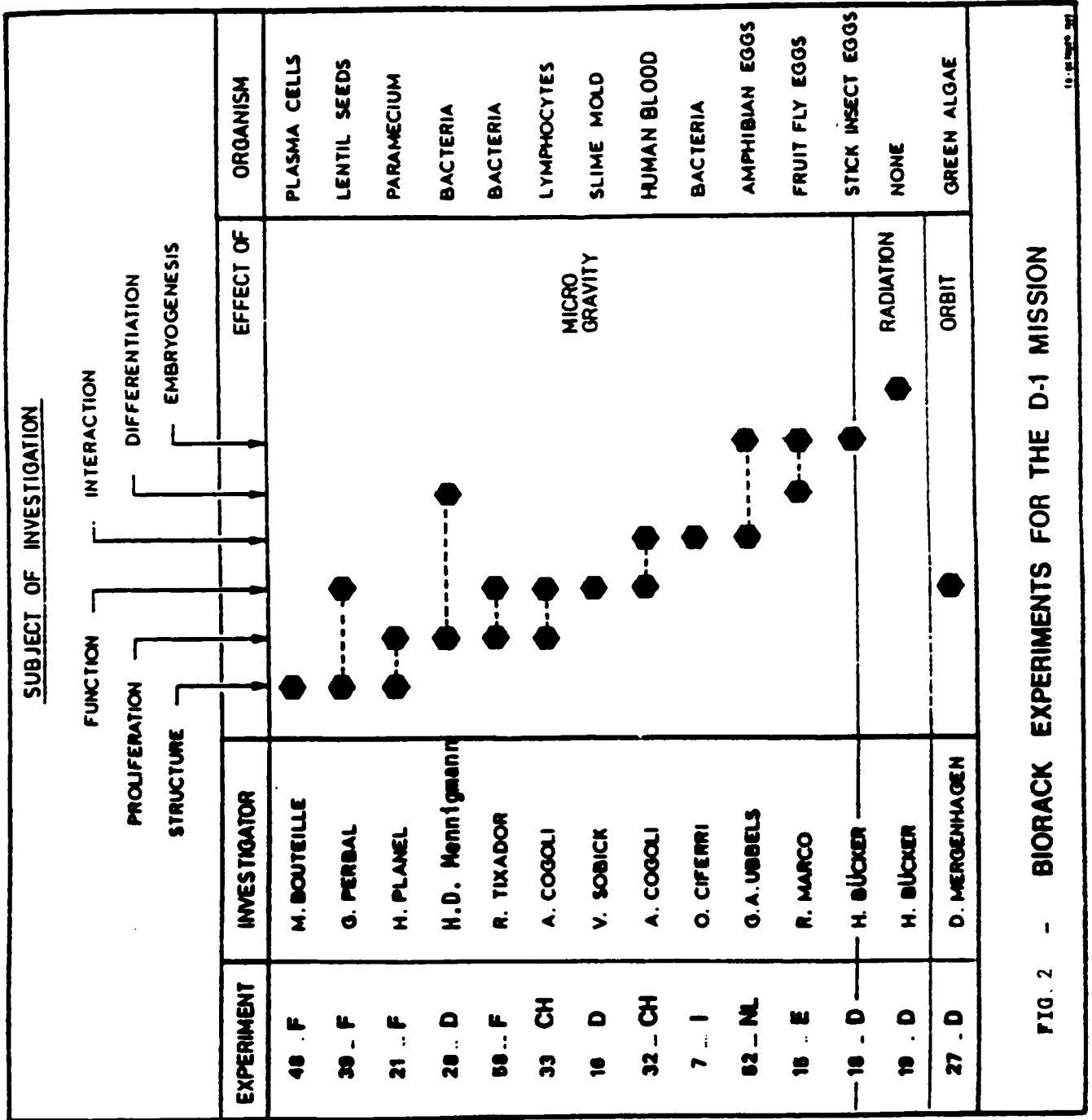
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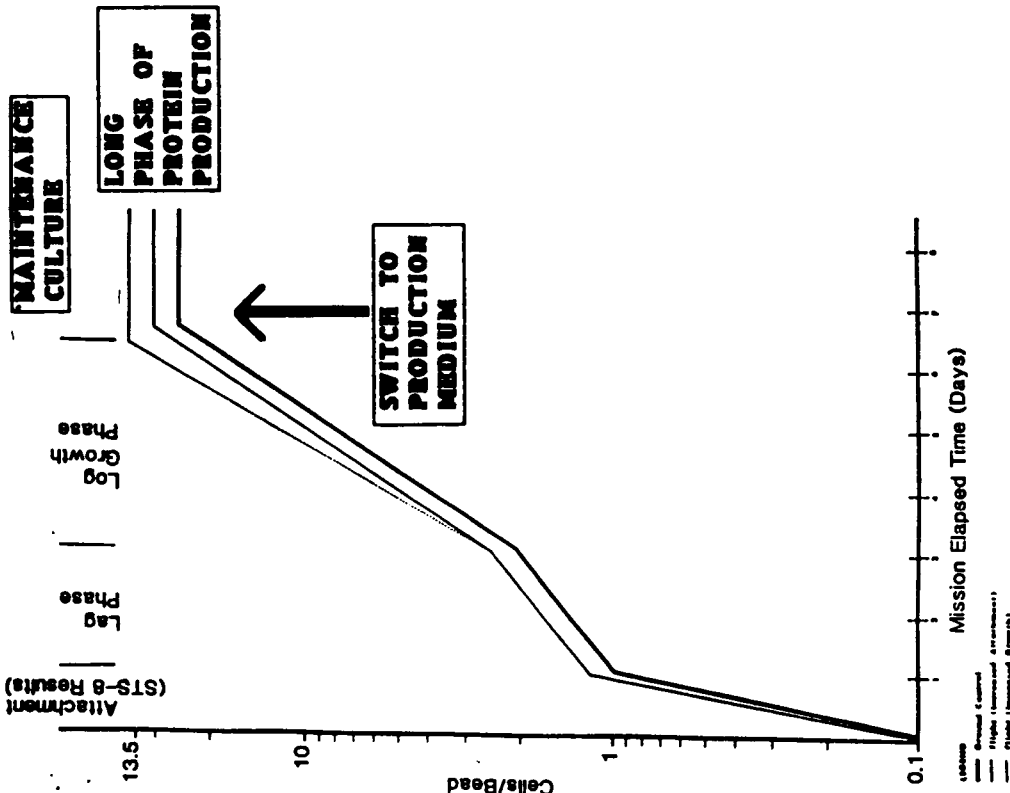
(Nov. 1985)

MICRO-G EFFECTS

ORGANISM	
D. malanogaster	no. of embyros layed was 240% more than 1-G(Earth) and 25% more than inflight control (1-G)
	no. of developed embryos was only 10-15% that of 1-G (Earth & inflight) decrease in life-span of males, but not females
E. coli	increased resistance to Colistin (antibiotic) 2 mg/l. vs 1 mg/l. at 1-G
E. coli	DNA transfer at conjugation was 2-3X higher transduction and transformation same as 1-G
B. subtilis	growth started earlier, final biomass greater, sporulation was less
Physarum sp.	increased velocity of protoplasmic streaming, periodicity unchanged
Paramecium tetraurelia	cell proliferation increased 4X (confirmed Salyut-6 & 7 results) cell volume increased and intracellular Ca was decreased
lymphocytes (human)	confirmed that ConA mitogenic response was decreased by more than 90% 60% decrease in 1-G inflight control vs. 30% increase at 10-G's
plasma cell hybridomas	ultrastructure. RNA synthesis. normal growth after return to Earth

FIGURE 3

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BIOREACTOR FLIGHT PROPOSALS

IN PREPARATION

1) KIDNEY CELL GROWTH - JSC IN HOUSE
   - INCREASED ATTACHMENT
   - HIGH DENSITY
2) LOW SHEAR EFFECTS - JSC, RICE, U.H.
   - CELL METABOLISM
   - CELL STRESS
   - PRODUCT FORMATION

OTHER PROPOSALS EXPECTED

- GROWTH OF PLANT PROTOPLASTS
  KRIKORIAN (S.U.N.Y.), SCHELD
- CULTURE IN BUBBLY MEDIA
  DUCKLER (U.H.)
- FRAGILE HYBRIDOMAS
  LEY (LOVELACE MED.)
- YEAST FERMENTATION
  PETERSON (JPL)
- BIOSYNTHESIS OF CELLULOSE
  BROWN (U.T.)

TYPICAL HUMAN CELL CULTURES

MAINTENANCE CULTURE

LONG PHASE OF PROTEIN PRODUCTION

SWITCH TO PRODUCTION MEDIUM

Attachment (STS-8 Results)

Lag Phase

Log Growth Phase

Cells/Bead

13.5

10

1

0.1

Mission Elapsed Time (Days)

Fig. 4