# IMMUNE CHANGES IN HUMANS CONCOMITANT WITH SPACE FLIGHTS OF UP TO 10 DAYS DURATION

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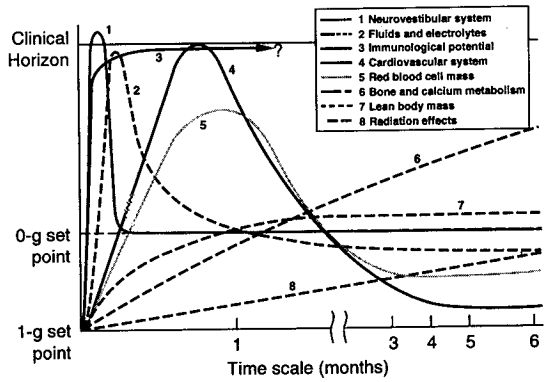
Space flight offers an important tool to life sciences researchers, while at the same time creating operationally important problems requiring solution for optimal crew safety and productivity. The time relation of various classes of in-flight human physiological changes may be illustrated with the histogram shown in the figure 1.

As this figure shows, certain problems, such as neurovestibular, fluid, and electrolyte imbalances tend to occur early in a flight followed by stabilization at some micro-gravity equilibration level. Cardiovascular dysfunctions and erythrocyte mass losses appear to follow a similar pattern, although the significant changes occur later in flight. Bone and calcium changes and radiation effects are thought to progressively worsen with time, whereas the time course of immune changes is yet to be fully understood (1).

**Time Course of Physiologic Alterations in Microgravity**




Over the past 20 years we have documented certain significant immunologic changes in cosmonauts and astronauts during or after space flight (2). Human immunologic changes reported from a variety of U.S and Russian studies include major depressions in the ability of blast cells to transform in response to mitogenic challenge, a loss of cytokine production or function, major changes in peripheral or splenic immune cell populations, alterations in natural killer cell activity and response to colony stimulating factor, and depressions in the delayed-type hypersensitivity response. The few studies that have been conducted with the antibody-mediated humoral immune system have been inconclusive. Although occasional post flight quantitative changes in immunoglobulin classes have been reported, the ability to normally produce specific antibodies *in vivo* in response to antigenic challenge remains to be tested. Therefore, the effect of space flight on the ability of the body to produce antibodies remains unexplored.

Microbiological changes have likewise been documented (3). These have include a "simplification" of crew autoflora, characterized by a significant reduction of saprophites, with a relative increase in the incidence of potentially pathogenic microorganisms on body surfaces. In addition, there has been a buildup of yeasts and filamentous fungi within the space cabin, microbial contamination between crew members, and increased "pathogenicity" of certain species following space flight.

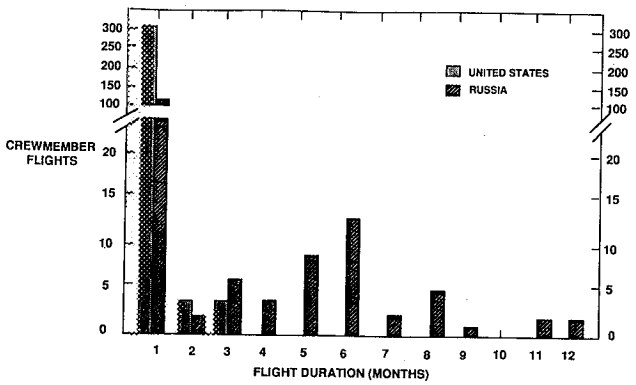
The above information indicates that space flight can be expected to effect a blunting of the human cellular immune mechanism concomitant with a relative increase in potentially pathogenic microorganisms. This combination would seem to increase the probability of infectious disease events in flight (4). In fact, there was a very high in-flight infectious disease incidence reported in the early days of the U.S. space program before adoption of a preflight health stabilization program. For example, during the first seven flights of the Apollo program illness events were not uncommon, with crew members experiencing upper respiratory problems, influenza, viral gastroenteritis, rhinitis, pharyngitis, and mild dermatologic problems (5). Apollo 13 was an especially important mission from the infectious disease point of view. First, one of the crew members was removed from flight just days before launch after being exposed to an active case of measles. Second, an active *Pseudomonas aeruginosa* urinary tract infection developed from the latent state in one crew member. It will be remembered that the Apollo 13 vehicle was partially destroyed while traveling towards the moon and that the remainder of the mission was characterized by unusually high stress. Given what we know about the blunting of immune capability during space flight there is no doubt that stress exacerbated this infection (6).

Preflight crew isolation was initiated following the Apollo 13 mission and has continued in one form or another as an integral part of the U.S. space program (7). This procedure was designed to allow the autoflora to equilibrate at a level consistent with confinement and to allow contracted infectious agents to demonstrate themselves before flight. It is likely that this procedure contributed to the significant reduction in microbial problems reported to have occurred during, or immediately following U.S. Space flights subsequent to Apollo 13. We do not have reliable data to show the effect of space flight on the immune system prior to Apollo 14, before the preflight health stabilization program was initiated. However, data collected subsequent to Apollo 13 show certain changes in immunological parameters, outlined below, that would be of greater concern without the intervention of this effective countermeasure.

Throughout the last decade the Russian and U.S. space programs have followed somewhat different paths as shown in figure 2. As these data reveal, the U.S. manned-space program has emphasized flights

of less than one month. In fact, except for those associated with the three SKYLAB flights, all U.S. astronauts have been in space for less than two weeks at a time. However, the Russian manned space program has incorporated many flights with varying length up to a full year. Therefore, this paper emphasizes immunological changes during "short-duration" missions. The "long-duration" results have been presented by Konstantinova *et al* (8).

**CREWMEMBER FLIGHTS UP TO 12 MONTHS DURATION**




Extensive comparisons of preflight and post flight immunological parameters were conducted with the first 41 U.S. Space Shuttle astronauts (9) as summarized in table 1. In this table is shown the number of times a particular assay resulted in a larger, or a smaller, post flight value when compared with the pre flight baseline for that individual.

**Summary of Postflight Changes in Shuttle Crew Peripheral Blood Cells[a]**

| Factor | n | NPI[(b)] | NPD[(c)] | APC[(d)] |
|---|---|---|---|---|
| Lymphocyte Number | 41 | 9 | 31 | -13.3 |
| Lymphocyte Stimulation | 41 | 5 | 36 | -25.7 |
| Neutrophil Number | 41 | 40 | 1 | +102.0 |
| Eosinophil Percent | 41 | 4 | 35 | NA* |
| Pan T Lymphocyte | 11 | 6 | 5 | +1.6 |
| Pan B Lymphocyte | 11 | 4 | 7 | +9.7 |
| Pan Monocyte | 11 | 3 | 7 | -11.6 |
| "T" Helper | 11 | 8 | 3 | +11.1 |
| "T" Suppressor | 11 | 5 | 5 | -2.3 |
| T4/T8 Ratio | 11 | 7 | 4 | +13.4 |

[a] From Taylor and Dardano (1986)
[b] Number of postflight increases
[c] Number of postflight decreases
[d] Average postflight change

* Eosinophil percent postflight change not a useful statistic because the count is typically reduced from small number preflight to zero postflight.

This study demonstrated unequivocally that the absolute number of lymphocytes in the peripheral circulation, the ability of these cells to respond to mitogenic stimulation, and the number of eosinophils in the peripheral circulation were typically decreased after flight. Conversely, there was an almost universal doubling of the absolute neutrophil number. Often there was a major change in the T4/T8 ratio, resulting from an increase in the Helper lymphocyte population. Additional data from 11 crew members, indicate a post flight decrease in circulating monocytes and "B" lymphocytes. Further, the reduced "T" lymphocyte blastogenesis was shown to correlate with the decreased monocyte count (9).

More recently, an additional group of 30 U.S. Shuttle astronauts were evaluated using similar methods (10). The results of this study are shown on table 2. The resulting slide cell differential data confirmed the customary granulocytic increase and lymphocytic decrease within the peripheral circulation post flight. However, contrary to previous findings this study reported a 52 % increase in the post flight monocyte population. This increase was borne out by a significant ($P<0.01$) increase in monocytes as derived from subset analysis of isolated peripheral blood mononuclear cells extracts as shown in table 2.

**Effect of space flight on peripheral blood leukocytes of 30 U.S. Astronauts**

| Test | Launch minus 10 days | Launch minus 2 days | Landing day | Landing plus 3 days |
|---|---|---|---|---|
| Total leukocytes | 5800 ± 200 | 5600 ± 200 | 7000 ± 200* | 5680 ± 200 |
| Granulocytes | 3200 ± 116 | 3024 ± 112 | 4970 ± 140* | 3080 ± 112 |
| Lymphocytes | 2262 ± 58 | 2240 ± 112 | 1680 ± 70* | 2128 ± 112 |
| Monocytes | 133 ± 17 | 190 ± 22 | 245 ± 28* | 190 ± 28 |
| Monocytes ($CD14^+$) | 13 ± 1 | 12 ± 1 | 21 ± 1* | 13 ± 1 |
| T inducer ($CD4^+$, Leu-$8^+$) | 32 ± 2 | 34 ± 2 | 23 ± 1* | 31 ± 2 |
| T cytotoxic ($CD8^+$, $CD11b^-$) | 18 ±1 | 16 ± 1 | 12 ± 1** | 17 ± 2 |
| T helper ($CD4^+$, Leu-$8^-$) | 5 ± 1 | 7 ± 1 | 7 ± 1 | 7 ± 1 |
| T suppressor ($CD8^+$, $CD11b^+$) | 3 ± 1 | 3 ± 1 | 3 ± 1 | 2 ± 1 |
| NK cells ($CD16^+$ or $CD56^+$) | 9 ± 1 | 9 ± 1 | 3 ± 1* | 5 ± 1* |
| B cells ($CD19^+$) | 7 ± 1 | 6 ± 1 | 6 ± 1 | 7 ± 1 |

After Meehans *et al.* 1992
Dara are mean ± SE of cells/$mm^3$ determined by slide whole blood differential
Data are mean ± SE of the percentage of MNC which express specific cell-surface antigens
NK assay performed on 10 astronauts samples
*$P$ <0.01 Landing versus launch minus 10 days and 3 days after landing.
* $P$ <0.05 Landing day versus launch minus 10 days, 2 days and 3 days after landing.

The authors of these data, Meehan *et al.*, have indicated that the apparent discrepancy may be the result of mission length. Crew members that demonstrated a post flight increase in peripheral blood monocytes were in space for 4 to 5 days (10). Conversely, those showing a post flight decrease in peripheral blood monocytes were in space for 6 to 8 days (9). These results suggest that the monocyte population moves between compartments as the mission progresses, up to 8 days. This progression may in fact be preceded by related neuroendocrine changes. Meehan *et al.* indicate that the noted increase in monocytes is inconsistent with the reported increase in glucocorticoids, since this should be accompanied by a decrease in peripheral blood monocytes. Likewise, the percentages of insulin receptor-positive cells and IGF-I receptor-positive cells did not increase following flight as would be expected with increased monocytes. Therefore, these researchers may have been fortunate enough to sample the population between a change in the neuroendocrine cause, and the immune cell response. In addition to the very

interesting monocyte findings, the data in this table also indicate the expected post flight reduction in the number of "T" inducer, "T" cytotoxic, and NK cells within the peripheral circulation.

The post-flight studies outlined above are very important because it is often operationally impossible to collect data in any other way, and they have given us important clues concerning immunological changes resulting from space flight. However, reliance on post flight analyses presents serious problems with data interpretation. Most important is the fact that the samples must be collected after the stressful conditions associated with landing, followed by some variable degree of reacclimation to terrestrial conditions (11). Thus, one can not, in this way, adequately separate in-flight from landing conditions as affectors of noted changes to the immune system. In addition, the length of time elapsing between when the space craft returns to Earth, and when investigators have access to crew members for sample collection or medical analysis varies with each flight. This time lag problem was somewhat alleviated with the advent of the U.S. Space Shuttle program because the shuttle vehicles optimally land on a pre-determined runway. From the point of view of scientific return, this is a great improvement over previous programs where the actual landing could be many miles from the anticipated site.

These post flight tests have typically been conducted *in vitro*. Therefore, a determination of the degree to which crew members were immunocompromized required extrapolation. This is no different than the situation one is typically presented with in health care. However, it has made statements about the clinical importance of the noted immune changes more difficult to support.

In a very few cases, an attempt was made to solve the problem of conducting tests on post-flight samples by collecting samples in-flight and either analyzing them post-flight or in-flight. In the case of post-flight analysis, sample storage conditions were found to be highly unpredictable and the results were generally not of much use. The few times that in-flight sample analyses were attempted demonstrated that the response to analysis conditions, especially cell culture, were greatly different in flight (12).

Only recently has the effect of spaceflight on the ability of the human cell mediated immune (CMI) system to function normally *in vivo* been tested in flight as shown in figure 3.

The ability of U.S. Space Shuttle crew members to mount a delayed-type hypersensitivity response was evaluated, in flight (13) with the Merieux Multitest Cell-Mediated Immunity (CMI) System. This system consists of a plastic skin puncture device that simultaneously injects seven different glycerinated recall antigens and one glycerine control in a standard pattern. Reactivity, reliability, repeatability, and safety of the antigens and application technique have previously been established through extensive field evaluations (14). Concentrations were selected such that each was the lowest possible which still produced the maximal incidence of positive DTH reaction in a representative population of normal healthy adults. This procedure is highly compatible to in flight testing because the incidence of large reactions is reduced, thus allowing for application sites to be placed only 20 mm apart. Also, by using a "minimal" concentration of each antigen, the sensitivity of the test for detecting hypoergy or anergy is maximized. We have established that the delayed-type hypersensitivity (DTH) response to common recall antigens is a simple, yet effective method for evaluating inflight-mediated hypoergy (13).

INFLIGHT CHANGES IN DTH REACTIONS OF TEN U.S SPACE SHUTTLE CREWMEMBERS

| | | No. of Positive Reactions | | Reaction Score (mm) | |
|---|---|---|---|---|---|
| Subject | Mission Length | PF[b] | IF[c] | PF[b] | IF[c] |
| 1 | 4 | 6 | 5 | 31.5 | 32.7 |
| 2 | 4 | 4 | 5 | 16.0 | 18.3 |
| 3 | 4 | 6 | 4 | 37.1 | 18.8 |
| 4 | 5 | 2 | 0 | 7.0 | 0.0 |
| 5 | 5 | 5 | 2 | 22.8 | 11.0 |
| 6 | 5 | 5 | 3 | 26.0 | 10.5 |
| 7 | 10 | 5 | 3 | 19.5 | 11.5 |
| 8 | 10 | 5 | 3 | 21.0 | 12.0 |
| 9 | 10 | 3 | 2 | 10.5 | 8.5 |
| 10 | 40 | 4 | 3 | 23.0 | 13.5 |

From Taylor and Dardano, 1986
[a] in days; [b] Preflight; [c] Inflight

The CMI mechanism was evaluated in ten astronauts by measuring their in-flight DTH response to the common recall antigens of Tetanus, Diphtheria, *Streptococcus*, *Proteus*, old tuberculin, *Candida*, and *Trichophyton*. The results obtained from each of the 10 crew members are illustrated in table 3. On all occasions except one (crew member 2) the cell-mediated immune system responded to

fewer antigens in flight as compared to the preflight response. It should be noted that crew member 2 was on the shortest flight tested. Crew member 4 was the only subject who demonstrated anergy during space flight. This subject was aboard the 5-day flight.

In-flight data were also analyzed according to the total value, in mm, of the mean induration diameters of all the positive reactions for a particular subject. This is referred to as the reaction score. In all but two cases (crew member 1 and 2) the reaction score was decreased during flight. Again, these two subjects that registered an increase were aboard the shortest mission. These results demonstrate that hypoergy was the least during the shortest (4 day) mission, whereas the 5-day mission resulted in the greatest change.

These data suggest that on day four of a Space Shuttle mission the cell-mediated immune system is measurably degraded and that between day 5 and day 10 the depression maximizes and the CMI mechanism begins to adjust to the new conditions. These findings would tend to support the previously-discussed monocyte data because monocyte control also appears to change considerably between day 4 and 5 of space flight. This similarity of results is very useful for developing an explanation of the mechanism of immune depression early in the mission since cells of the macrophage lineage are generally considered to be the main antigen-presenting cells in the DTH reaction.

*In vivo* T cell proliferation requires interferon gamma (INF-g) and probably interleukin 1 (IL-1). In addition, proper secretion, and activity of interleukin 2 (IL-2) is necessary for feedback control between lymphocytes. Significant decreases in interleukin production (especially IL-2) and interferon (INF) alpha/beta and gamma have been reported in Cosmonauts as shown in table 4.

**Capacity for Interferon-Formation of Lymphocytes in the Peripheral Blood of Cosmonauts after Short Flights on Salyut 6 and Salyut 7 Orbital Stations**

| Orbital Station | Expedition | Duration of Flight (Days) | Cosmonaut | Activity of IFN-α, IU/ml* | |
|---|---|---|---|---|---|
| | | | | 30-45 Days Before Flight | One Day After Flight |
| Salyut-6 | EP 4-1 | 8 | 1 | 48 | 6 |
| | | | 2 | 32 | 8 |
| | EP 5-2 | 7 | 1 | 4 | 4 |
| | | | 2 | 6 | 4 |
| Salyut-7 | EP 1-1 | 8 | 1 | 80 | 10 |
| | | | 2 | 10 | 10 |
| | | | 3 | 160 | 10 |
| | EP 1-2 | 8 | 1 | 80 | 40 |
| | | | 2 | 40 | 40 |

From: Konstantinova, 1988

These data show a decreased ability to elaborate interferon alpha in blood collected post flight from 6 of 9 Russian Cosmonauts. In the future, a thorough in-flight investigation of T-lymphocyte and monocyte activity is essential to determining the degree to which interleukins contribute to the identified cell-mediated immune dysfunction. Such investigations should include, but not be limited to:

[1] IL-1 mediated activities such as prostoglandin production, activation of natural killer (NK) cells, macrophages, and lymphocytes
[2] the balance between IL-2 receptor activity and IL-2 production
[3] INF-gamma production by activated T lymphocytes and NK cells.

Finally, in the future it will be important to analyze the in flight immune system results both in light of neuroendocrine data and reliable estimates of the stress environment experienced by each crew member. Only in this way can the influence upon the immune system of microgravity-induced changes throughout the body be determined.

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