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Appendix A

Some comments on biological aspects of life support systems

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A.1 Introduction

In this Section we discuss, in outline, some of the aspects of the space life sciences that relate to the problems of extended manned space missions. This is not intended to be a fully detailed review, but rather will indicate some of the relevant literature by describing selected research results and introducing the main problems that still exist and need to be addressed.

A.2 Bioregenerative life support systems

Bioregenerative life support systems (BLS) are intended to recycle CO_2, waste, including faeces, and to produce oxygen and edible biomass. Principally, the systems are composed of several interconnected compartments which form a more or less closed loop. The recycling component mainly consists of microorganisms, whereas other compartments differ in the complexity of their constituents (from enzymes to plants and animals). For the International Space Station, a facility to grow fresh vegetables on a continuous basis (so-called 'salad machine') is foreseen and can be considered as a step towards the development of more complex BLS which serve aesthetic and psychological, as well as nutritional, purposes.

Both recycling and production must be maintained reliably for long periods of time. The interdependency of BLS compartments renders them subject to system instability if even a single compartment fails, so any perturbation in one element may affect the whole system. Dynamic changes within the system may, on the other hand, lead to self-regulation if internal or environmental factors vary. Technological control, essentially based on selective handling of flows between compartments, must prevent damage becoming irreversible and potentially endangering the whole system.

Reliable, solid, and robust technology; high productivity and low waste production; low maintenance, high automatization; and compatibility with microgravity conditions, are the most important criteria for BLS. Stability and redundancy are additional valuable features.

The substances participating in BLS reactions may be grouped as substrates, products, catalysts, and inert matter. Products of one compartment may serve as substrates in another so that in complex interconnections there is a network of bioprocesses in which individual pathways may be temporarily reversed for the sake of integrated system functioning.

Within the compartment generating them, substrates often display significant inhibitory action on catalysts (feedback inhibition) and hence must be eliminated or kept at an acceptable level. Undesirable compounds (like toxic gases, proteins inducing foam formation, polymers increasing viscosity) need to be closely monitored.

Life support systems are generally classified as physicochemical or bioregenerative; however, the former quickly become biological due to microbial growth which in certain instances turn out to be beneficial. As an example, water purification systems using granulated activated carbon rapidly become colonized with bacteria. This was actually found to increase efficiency of removal of particular organic contaminants and also to extend the effective life of the purification system.

Recycling is the main criterion of BLS, whereas *provisioned* systems need *reclamation*, which means that consumables have to be regularly restored to acceptable quality standards prior to reuse. Partially closed BLS need relatively little reclamation; most systems vary over time with respect to their degree of closure.

Source and sink size are critical determinants in the design and specifications of BLS. Evidently, an outside energy source is always needed to keep the system alive. A sink is any element (or organism) which functionally removes any substance from the larger system. This can be achieved through biochemical transformation to compounds more acceptable to the recipients, and/or through trapping and storing for later removal.

It has been demonstrated that a system with larger sinks is more flexible and allows design specifications to be less precise. The largest test bed so far is Biosphere-2 in Arizona, with more than 3000 species interacting as a broad network of interdependent sinks. The finding of an unexpectedly active oxygen sink in the soil, which then required that oxygen be injected into the system after little more than 1 year's operation, demonstrated how much is still to be learned about complex BLS. Self-regulation is a common feature of natural ecological systems, but stability cannot be taken for granted. Even our planet, the BLS with the largest sinks known, has bioregenerative limits. Therefore, the sheer magnitude and complexity of a BLS does not necessarily guarantee long-term stability.

Colonization of future space settlements (Moon, Mars) will inevitably call for stable, reliable, and complex BLS; and to arrive at this goal, much more research will be necessary to get sufficient knowledge on the critical points within a complete BLS. Biosphere-2, in particular, is an unique test bed having sufficient magnitude for this type of investigation, but no matter how impressive such achievements appear, some enormous problems in basic biology remain. There is a small but active group of people carrying out research on various aspects of BLS and CELLS (closed ecological life support systems). Atmosphere recycling being a prime design criterion, a variety of plants are being studied for their potential as atmospheric 'scrubbers'. These range from algae, through water weeds (e.g. *Lemna* spp. - the duckweeds), to established crop plants like wheat and potatoes. This is not the place for a detailed review. The topic was dealt with by MacElroy *et al.* (1985) and in a relatively recent COSPAR Symposium (published in *Advances in Space Research*, volume **9** (part 8), 1989).

What needs to be emphasised here is that whilst constructions like Biosphere-2 and the Soviet BIOS-3 demonstrate that completely sealed closed ecological systems can be made to work on Earth, we are still too ignorant of the effects of long term exposure to space flight conditions to be confident that similar systems would continue to work in space. On Earth, microgravity can be simulated (parabolic flight, drop towers) for such short periods of time that they are simply not applicable to testing BLS. Neither can the radiation pattern encountered in space be simulated on Earth. We quote Krikorian & Levine (1991):

> "...CELSS-related activities directed towards finding practical solutions for life support in the space environment are dependent on basic science. Any CELSS effort aimed at solving practical problems and providing practical solutions to difficulties encountered in growing plants in space will of necessity rely on the answers generated by basic research performed by gravitational plant biologists..."

To this statement we would add the fact which is stressed in Chapter 1 (Section 1.6): that only one plant and one insect have been taken through a complete generation cycle in orbit. Both exhibited a high frequency of developmental abnormalities in the progeny produced in microgravity that were of a sort which could adversely affect the ability of those progeny to form a further generation. Since no multicellular animal or plant has yet been taken through more than one generation in orbit, the stability of even single populations, let alone mixed, competing populations in an ecological microcosm, is seriously in doubt.

A.3 Food, nutrition, metabolism

A.3.1 Background

Only limited data are available on the adaptive role of metabolic readjustment in man during spaceflight. With increasing mission duration and distance from earth, there is a corresponding increase in the scientific and logistical problems of ensuring adequate nutritional supply. The range of available food and drink must guarantee that all essential nutrients are supplied in the correct balance to maintain the physiological range of life functions and well-being under the conditions of working and living in a spaceship or extraterrestrial habitat. The database available today from experiences collected so far does not allow for precise projections of nutritional problems which are likely to arise during missions of extended range from Earth, like flights to Mars. Adaptive changes which improve human performance and well-being in space might have detrimental consequences upon return to Earth (Seddon *et al*., 1994).

Inadequate nutrient supply to the tissues results in nutritional deficiency. Human diet is composed of macronutrients - water, proteins, carbohydrates, fat, nucleic acids - and micronutrients - trace elements and vitamins - which regulate metabolic processes by acting as coenzymes or as essential elemental constituents of enzyme complexes which regulate the utilization of proteins, carbohydrates and fats. It is conceivable that future long-term flights will require fine balancing of the nutritional adequacy of the diet.

From the 20 amino acids which are supplied by dietary protein, 8 to 10 are essential in the diet of humans because they cannot be synthesized. The requirements of essential amino acids range from 3 to 15 mg kg^{-1} day^{-1} in adults. Animal protein generally provides good proportions of essential amino acids (Linkswiler 1982). The recommended daily allowance (RDA) for protein, assuming a mixture of animal and vegetable protein, is about 60 g day^{-1} for men, and about 50 g day^{-1} for women.

Dietary protein intake influences albumin metabolism (James & Hay 1968), blood volume, fluid balance, cardiovascular functioning, and body composition. A major microgravity-related effect is deconditioning of the musculoskeletal system, which is made evident as atrophy of 'antigravity' muscles, with loss of muscle mass (and nitrogen). Bodily fat stores are also usually diminished (Forbes 1987), and decreased mechanical load is thought to result in bone catabolism as well, with consequential loss of protein and calcium (Stein & Gaprindashrili 1994).

Nutritional fat is a vehicle for flavours, fat-soluble vitamins, gives texture, delays gastric emptying, and cushions body organs (Coniglio 1984; Flatt 1987). The caloric value is the highest of all nutrients. Fat consumption furnishes essential fatty acids; the needs can be met by a daily intake of 15-25 g of proper dietary fat. Fat is of primary importance for buffering energetic needs: day-to-day fluctuations in energy balance is met primarily by body fat rather than by carbohydrate or protein (Abbott *et al.* 1988). A dietary source of polyunsaturated fatty acids is required by humans; these are linoleic acid, α-linoleic acid, and arachidonic acid. The amount of linoleic acid required by humans is about 1-2% of dietary energy; linoleic acid is a prominent component of the dietary fats. Essential fatty acids and their derivatives are precursors of prostaglandins, thromboxanes and prostacyclins. They are involved in membrane structures and play a beneficial role in cardiovascular and blood pressure regulation (Iacono *et al.* 1982).

Carbohydrates are not essential in the sense that essential amino acids or fatty acids are, but digestible carbohydrates are the most important source of food energy, and indigestible polysaccharides contribute to dietary fibre. The brain and the complement of red blood cells, which depend almost entirely on carbohydrates for energy metabolism, together consume 150-200 g glucose d^{-1}. About half of dietary energy is provided by carbohydrates.

This corresponds to 5 to 6 MJ metabolizable energy from 300 to 350 g digestible carbohydrate; a minimum of about 2 g kg^{-1} (normally about 150 g) is required to prevent ketosis.

The main source of carbohydrate, as well as the only source of dietary fibre, is vegetable food. Foods which are rich in starch also contain dietary fibre and a variety of essential nutrients. Consumption of fibre is desirable from a preventive medical point of view because it increases stool mass, decreases turnover time of bowel contents (Eastwood 1984), and binds potential carcinogenic compounds. The effect on stool weight may be directly due to water-holding capacity (fibres resist mucosal absorption of water), or indirect as a result of bacterial growth or increase in metabolites (e.g. volatile fatty acids). Fibre is subject to bacterial decomposition in the large intestine and gives rise to flatulence; dietary fibre in space food requires careful consideration.

With regard to minerals, the estimated safe and adequate daily dietary intake of sodium is 1.1 to 3.3 g in adults. Sodium intake is best assessed by urinary sodium output. Usual daily potassium intake ranges between 3 and 11 g. A daily minimum need of 1 g can be reasonably assumed; estimates range from 0.8 to 3 g d^{-1}. With usual diet, there is no danger of insufficient supply of potassium. Calcium constitutes 1.5 to 2% of the total body mass, with >99% present in bones, where the ratio to phosphorus is somewhat greater than 2:1. About 85% of body phosphorus (0.7 g) is in bone as calcium phosphate and hydroxyapatite, 15% in cells and extracellular fluids. In the usual diet, the phosphorus:calcium ratio is high, e.g. 15 to 20 in meat, about 2 in eggs, grains, and nuts. In contrast, most green leafy vegetables contain more calcium than phosphorus. The recommended Ca:P ratio of 1:1 is impossible to achieve in diets high in protein.

A multitude of trace elements are essential for life, serving as cofactors in enzyme reactions, binding of oxygen, and structural components of non-enzymatic macromolecules (Linder 1985). The number of indices that are presently available to assess accurately the status of the majority of the trace elements is limited. Circulating levels may not reflect the status of the element available for nutritional needs.

Tissue stores of a trace element may not be available to meet the needs during deficient supply because they may be bound to enzyme proteins from which cellular stores cannot be mobilized. The action of trace elements depends upon nutrition, metabolism, age, and gender. Trace elements play an essential role for immunity; immobilization, isolation, weightlessness, and/or altered gravitational acceleration may decrease defence capabilities *via* altered trace element status. A particularly important role may be played by nutritional fluoride (Boivin *et al*. 1993) in long-term flight.

Iron status is best assessed by measuring plasma ferritin which normally ranges from 20 to 200 μg l^{-1}; each μg l^{-1} corresponds to 140 μg kg^{-1} BW of iron stores (Finch & Hubers 1982). Zinc status may be assessed by leukocyte zinc levels (Bro *et al.* 1988), or erythrocyte Cu, Zn superoxide dismutase, and serum alkaline phosphatase activity (Hambidge *et al.* 1989). Copper status seems to be indicated by the specific activity of ceruloplasmin, as estimated by the ratio of enzymatic to immunoreactive ceruloplasmin, erythrocyte Cu, Zn superoxide dismutase, or saliva copper concentration (Milne *et al.* 1990; Bales *et al*. 1990).

Selenium is measured in whole blood and plasma, erythrocytes, platelets and leucocytes; plasma and platelet glutathione peroxidase is sensitive to selenium intake and can be used to assess selenium needs (Sandström *et al.* 1990). Urinary selenium excretion is used to estimate selenium intake. Serum and urinary *manganese* concentration is not sensitive to dietary alterations (Greger & Davis 1990), but probably reflects manganese status.

Vitamin A (retinol) is essential for vision, growth, epithelial cell differentiation, reproduction, and the integrity of the immune system. Retinol also influences calcium metabolism and calcification. Various carotenoids are biologically active as vitamin A.

Vitamin D (D-hormone) and its metabolites regulate calcium, phosphorus, and bone metabolism. Parathyroid hormone causes formation of the active hormone in the kidney. D-hormone stimulates active transport of calcium across the small intestine. Vitamin E (tocopherol) serves as an antioxidant, preventing (per)oxidation of polyunsaturated fatty acids in cell membranes, may play a role as anticarcinogenic as well as erythropoietic factor, and is thought to slow down aging processes (Linder 1985). Vitamin C (ascorbic acid) is an antioxidant as well, affecting the body's redox potential, and is involved in hydroxylation reactions, procollagen synthesis, and transport and absorption of iron. It enhances the response of neutrophils to chemotactic stimuli and also may exert other positive effects on the immune system.

A.3.2 Earth-gravity and simulated microgravity

A major factor in space flight nutrition is connected to deconditioning, catabolic state, and calcium loss. There is controversy how much calcium is needed for maintaining body stores, i.e. bone mass. On the basis of calcium balance studies conducted with groups of individuals accustomed to adequate intakes of foods high in calcium, the daily allowance is 800 mg for individuals 18 years of age and older (Lutwak 1982). Absorption of calcium from food sources is not influenced by solubility, it is determined mainly by other food components (Heaney *et al.* 1990), and is, besides hormonal influences, decreased by stress and immobilization. Vitamin D increases the gastrointestinal calcium absorption (Orwoll *et al.* 1988), underlining the significance of sufficient vitamin D intake in astronauts. The 0.8 g d^{-1} given to Shuttle astronauts may be insufficient and could be increased to 1.2 to 1.5 g d^{-1} in supplements or calcium-rich foods; this is a level beyond which additional calcium is not usually absorbed. However, this may raise concerns about hypercalciuria and possible renal stone formation.

Experimental studies on the requirement for calcium, phosphorus, and vitamin D in relation to hormonal reactions (e.g. calcitonin) and bone metabolism could be useful; phosphate intake would have to be standardized in this connection, because of possible depletion effects on sodium and zinc. If calcium supplements were provided on a continuous basis, e.g. starting 2 weeks prior to flight, urinary/faecal excretion studies would show which percentage of dietary calcium is retained by the body under circumstances of space flight.

Both in simulation studies and during prolonged space flight, the renal loss of calcium and potassium reaches a maximum by the second month, most probably caused by a reduced capacity of tissues to retain electrolytes. This phenomenon of diminished mineral pool can be attenuated but not completely avoided by the use of countermeasures, like exercise, LBNP, and salt-water supplement (Gazenko *et al.* 1982; Grigoriev 1983). It is an open question whether reduced gravity as on Mars ($0.4 \times g$) or Moon ($0.16 \times g$) is sufficient to prevent bone loss and if supplements in calcium, phosphorus, vitamin D, fluoride, or hormones will be needed in addition to exercise countermeasures during long-term stay on extraterrestrial bases.

About 60% of body magnesium is located in the skeleton. More than 300 enzymatic systems are magnesium-dependent, such as ATP-dependent enzyme reactions. Magnesium plays a key role in neuromuscular transmission and activity; it is essential for the normal metabolism of calcium and potassium in adult humans, and for the mobilization of calcium from bone (Shils 1985). It is the mineral ion of chlorophyll, and green vegetables are an important source. The intake of magnesium varies widely, the average range is probably 180 to 480 mg d^{-1}, the recommended daily allowance being 300 to 350 mg for adults. Two-thirds of ingested magnesium is excreted in the stool. A diminished extracellular Mg concentration may lead to dysfunction of excitable tissues within the neuromuscular and cardiovascular systems giving rise to tetany and neurological disorders. Serum magnesium indicates extracellular Mg status but not total body magnesium. Peripheral mononuclear blood cell Mg seems to be a good indicator for total body stores (Hook *et al.* 1987).

Trace elements are absorbed by the gut to a very different fraction (1 to 100%) of what is present in the diet. Dietary input of any nutrient may considerably exceed the fraction actually available to the body. The efficiency of absorption mechanisms correspond to the body's needs and metabolic status. Bioavailability also depends on the presence of other substances in the food. The mechanisms of digestion, absorption, and metabolism may be disrupted by physiological stress factors, such as motion sickness, nervousness and anxiety. Eating while agitated, fatigued, or worried may give rise to gastrointestinal disturbances. Nutrient utilization depends upon a great number of psychological and neurohormonal control mechanisms, the quantitative interaction of which needs to be studied in more detail, and with use of novel laboratory as well as mathematical simulation techniques.

A.3.3 In flight experimental evidence

Space diets have been designed according to the recommended daily allowance (RDA) values as published by the NRC's Food and Nutrition Board. The RDAs were developed for terrestrial use, and the actual needs of astronauts may differ, at least in terms of catabolic shifts due to bone/muscle degradation in microgravity. Energy and protein supply have been gradually increased in space diets, the highest figure being reached with the Voskhod cosmonaut's diet containing 3600 kcal d^{-1}.

Most space food today is dehydrated to reduce mass and transportation costs. Dehydration of solid food results in a mass/volume reduction by a factor of 3 (meat) to 10 (components extremely rich in water) due to the removal of solvent or bound water. Eggs may be compacted to about 30% of initial volume, potatoes to about 25% initial volume and fruit to 20-25% of initial volume.

During space flight, loss of body protein occurs through muscle atrophy (Ushakov 1980); physical activity is not only the major variable affecting caloric expenditure but also determines the nutritional need for amino acids. In the Apollo and Shuttle missions, protein consumption was about 80 g d^{-1}, based on ingredients used and on amino acid analyses. Great individual differences were observed (up to 160 g d^{-1}). The Russians have prescribed a higher proportion of protein, in an effort to counteract muscle degradation. For U.S. astronauts, protein provided 19% of nutrient energy (Leonard 1982; Popov 1975; Sauer & Calley 1975), and 23% in Soviet cosmonauts (Popov 1975). After Salyut missions, a decrease in amino acids was found in blood plasma, particularly in essential amino acids, and it has been concluded that astronaut diets should be supplemented with certain amino acids before, during and after flight (Popov & Latskevich 1984).

Fat intake of U.S. astronauts (Gemini, Apollo, Shuttle) decreased in flight, compared to the consumption on Earth (Altman & Talbot 1987). Data from Apollo, Skylab, and STS suggest that the energy costs of vigorous exercise such as EVAs are consistent with those associated with comparable physical activity on the ground. An assessment of fat mass by different methods indicated that in three Skylab flights, fat loss contributed 45% to total body weight loss, and lean mass 55% (Leonard *et al.* 1983).

Carbohydrate consumption was higher in astronauts in flight (averaging 400 g d^{-1}) than under control conditions (350 g d^{-1}) whereas crude fibre intake was the same in flight and on the ground (5 to 10 g d^{-1}). During Gemini, Apollo, and Skylab missions, a deterioration of glucose tolerance in flight was observed (Leach & Rambaut 1977), and with the first Shuttle missions, postflight blood glucose increased more than 20%, with great differences between individuals. Data should therefore be considered on an individual basis rather than as mean values in future investigations.

As an average figure from Skylab, a net loss of approximately 100 mmol of sodium (corresponding to 0.4% of the overall body stores) occurred from the extracellular space, stabilizing at a lower level after initial disturbance in flight (Leach *et al.* 1975; Leonard 1985). Evidence from Gemini 7, Apollo 15-17, and Skylab (Leach & Rambaut 1977) showed that body *potassium* stores steadily decrease in flights exceeding one month in duration; e.g. by 240 mmol in 30 days, representing a 0.7% diminution of the body stores despite adequate potassium ingestion throughout these missions (Leonard *et al.* 1983).

In weightlessness or with reduced gravitational load, there is decreased mechanical stress in the trabeculae of the lower body bones. This is not balanced by sustained or even increased mechanical demand in the upper parts of the body. Despite some net shift from the legs to the bones of the skull, neck, and arms, the calcium content in the astronaut's body decreases continually even after several months in flight. The negative calcium balance (high urinary output which is not met by equivalent calcium resorption from the gut) cannot be compensated by increased dietary calcium supply. Blood plasma calcium concentration is significantly elevated in flight, from 9.5 to 10.2 mg dl^{-1} (Leonard *et al.* 1983). Urinary calcium excretion is increased about two-fold after several weeks mission duration. The renal excretion of phosphate was found to be increased in astronauts by some 20%, due to continuing reduction in bone mass. Some decrease in serum magnesium has been reported post flight, evidence of significant in flight magnesium change seems to be lacking.

In U.S. space missions, vitamin A intake exceeded the RDA by up to 200% (Altman & Talbot 1987). Vitamin D was provided to 100% RDA in U.S. space foods by multivitamin supplements. This was considered advisable considering the in flight absence of UV light. In the Shuttle OFT programme, 15 IU d^{-1} vitamin E (about 15 mg tocopherol equivalent) was provided by the standard menu.

Evidence indicates that high dietary protein induces calcium loss (Hegsted 1981; Kim & Linkswiler 1979; Linkswiler *et al.* 1981; Schuette & Linkswiler 1984; Zemel 1988) as it may be desirable for space menus. Commonly used complex dietary proteins do not have these effects in strictly controlled long term human studies (Spencer *et al.* 1983) in which large amounts of dietary protein with a high phosphorus content were used. An average balance on protein, calcium, and phosphorus intake (Spencer *et al.* 1988) may therefore be desirable for space travellers.

A.3.4 Conclusions

Future long term exposure of humans to reduced gravitational acceleration will show nutritional and metabolic adaptation to microgravity conditions. The kinetics of protein and amino acids in the body need investigation in flight to understand properly the underlying adaptive mechanisms. It is reasonable to assume that muscle adapts immediately to the reduced work load in microgravity by losing protein. The hormonal background is still far from being understood. Insulin resistance occurs, as it does in ground-based studies of muscular wasting due to bed rest (Shangraw *et al*. 1988), resulting in decreased glucose uptake. Growth hormone activity also seems to be depressed during space flight (Sawchenko *et al*. 1992). The etiology of protein loss is most probably multifactorial and includes stress responses, under-loading and under-nutrition.

The balance of catabolic and anabolic pathways in bone and muscle depends upon the amount and time-profile of mechanical load. This load can be seen to be composed of basically two factors: Acceleration forces acting on the body as a whole (gravitational load); and resistant forces to be counteracted (movement, isometric exercise). The combination of these two factors determines the metabolic balance and nutrient demand of the musculoskeletal system. A reduction of the average forces to which this system is subjected leads to atrophy which cannot be counteracted by nutritional measures alone. Mechanical devices, such as the 'penguin' suit, seem to be effective in preventing protein catabolism and muscle atrophy. A loss of 1000 g muscle tissue would constitute a 200 g loss of protein, since muscle is 20% protein. There is a need to relate the demands of physical performance in space with preservation of muscle mass; dietary strategies can assist in preventing amino acid and protein loss from the astronaut's body.

Purified proteins and amino acid mixtures as they are used in experimental diets lead to calcium loss in humans and could theoretically exaggerate the problem of negative calcium balance with long lasting periods of microgravity.

It is still not known how disuse osteoporosis during space missions might be prevented, nor what the steady-state level of bone calcium might be after long duration flights. Dietary countermeasures, i.e. oral calcium supplements, are limited for several reasons. Absorption is regulated according to actual needs which are determined by local mechanic stress and strain in the bone; calcium overloading may result in formation of calcium-containing kidney stones, and calcium supplementation interferes with absorption of other minerals and trace elements.

The mechanisms of nutrient transfer from the intestines to the blood are functionally modified by the body's metabolic status, so the degree to which a nutrient is available for absorption and assimilation depends on the physiological condition of the body. Matrix effects influence the degree to which nutrients (mostly minerals and trace elements) are taken up by the intestinal mucosa. The resulting problem of reduced bioavailability may become important in long-term space travel if diets become imbalanced. Fluoride is required for proper functioning of bone and may be a candidate for possible decalcification countermeasures. Careful dosage is essential because chronic fluoride load exceeding demand by a factor of about 10 results in toxicity symptoms. The net result showed increased skeletal mass and decreased negative calcium balance. There are conflicting results on dosage and duration of the supplementation with or without combination with calcium administration. Investigations are needed to define optimal fluoridation in astronauts because no countermeasure yet applied were sufficient to prevent weightlessness induced bone loss. Manganese is linked to central nervous system function, so investigations into manganese balance in astronauts will be mandatory in extended missions; no information on metabolism of this important trace element in flight is yet available. Another interesting question is whether ascorbic acid, a vitamin which is nontoxic even with very high dosage (up to 100 times RDA), would provide increased immunological defence capacity as a countermeasure to possibly decreased immunological capability in astronauts during flight. Vitamin C (and B) was present in U.S. space menus at almost 10 times RDA, following findings of low blood concentrations of vitamin B and C after various stresses.

Another important problem is which lighting system will be employed in future space habitats in order to meet biological needs. Unless there is full-spectrum light or quartz windows to permit penetration of solar UV rays, it is likely that there will be insufficient endogenous vitamin D production. Vitamin D has special significance in the space environment because it is a primary regulator of calcium absorption. The amount of vitamin D required to alleviate insufficiency needs to be determined. At the same time, toxic levels should be considered; a safe range of exogenous vitamin intake on long term missions has not yet been established.

Thiamin status requires close monitoring in any long lasting mission; it can be assessed by measuring renal vitamin excretion and the transketolase activity in erythrocytes. Similarly, cobalamin (vitamin B-12) will probably have to be supplied by self-contained biological life support systems in order to avoid symptoms of deficiency. Macrocytic anaemia is one of the early consequences of low folate supply. Inter-relationships of the B-complex vitamins are essential in the performance of metabolic and catabolic reactions in the body, thus B-vitamins were generally supplied on a high level in U.S. space menus. The 10-

15% decrease in the astronaut's red cell mass prompted the recommendation of a folic acid supply of 200% RDA in flight. The OFT Shuttle standard menu supplied 400 µg folate which is identical with RDA folate intake. It is yet to be decided whether higher folate in astronaut diets will boost red cell synthesis, and if so, if increased erythropoiesis is beneficial in terms of cardiovascular adaptation to weightlessness. It might be hypothesized that on the level of blood viscosity and microcirculatory exchange, the adapted state of 'space anaemia' provides more advantage than a 'normal' red cell mass.

Space diets may be designed according to commonly recommended nutrient intake values which were developed for terrestrial use. Actual needs of astronauts may, however, deviate significantly from these general guidelines. The major differences between weightless and 1 × g conditions as they influence metabolic needs are in the cardiovascular/fluid electrolyte and musculo-skeletal systems. For astronauts, it is particularly important to maintain an optimal fluid and electrolyte balance. The need for potable water depends on the net fluid balance of the body which differs from ground control during space flight. The physiology of fluid volumes and electrolyte concentrations during space flight is closely connected to the regulation of energy metabolism, biomechanics, bone remodelling, endocrinology, kidney physiology, and cardiovascular functioning. There is still a lack of understanding how body subsystems interact quantitatively along the time-line of microgravity adaptation and 1 × g readaptation processes.

For long term space missions, it will be necessary to completely regenerate life support materials; as space habitats get farther from earth, resupply of food and nutrients will become increasingly difficult. The design of any such system has to be based on the very wide knowledge of food science and technology. In order to minimize the production load and waste treatments, a maximum 'index of nutritional quality' needs to be achieved. Nutritional quality and consistency may be optimized with respect to any stress conditions or crew requirements which have to be specified on an individual basis. To date, no database exists on features of such systems on sufficiently reliable quantitative grounds.

Food acceptance is of major importance in any situation where humans are in a remote and isolated, challenging situation since mood and motivation heavily depend on the hedonistic, rewarding character of eating. There are enormous research needs to be met before questions of nutritional support under space flight and extraterrestrial conditions are defined and can be answered clearly. Food of plant origin will probably contribute to a major part to the menus. Cereals, vegetables, fruits, peas, beans, lentils, nuts, and seeds will likely be the cornerstones of future space diet. Unless food of animal origin is added for longer missions (i.e. months or years), nutrient deficiencies may occur. Data on amino acid content of different edible proteins can be used for planning of balanced protein supply with vegetarian diets. Deficiencies may occur with a prolonged vegetarian diet, e.g. with calcium, iron, zinc, and other trace elements. Careful planning of balanced diets can avoid long term nutrition problems even with minimal sources of animal food added to the diet. Any combination of food, resulting in certain diets, should be monitored continuously for all essential nutrients. This can be done by using computer software which not only analyzes meal composition but also allows for automatic checking if individual needs are met by certain intake patterns. Such type of supply monitoring needs to be complemented by regular nutritional assessments.

A.4 Countermeasures

A.4.1 Background

The body adapts to weightlessness by musculoskeletal and cardiovascular deconditioning; unlike other physiologic adaptations, calcium loss occurring in the weight bearing bones during microgravity does not reach a plateau during the first 6 or so mission months.

Cosmonauts who have remained in space for extended time periods required several weeks of recovery time on Earth to return to their preflight physical health (Gazenko *et al.* 1980). It is not known whether longer stays in microgravity will result in any type of permanent damage either to the cardiovascular or musculoskeletal system.

Artificial gravity has been proposed as the most effective way to prevent deconditioning for long duration missions (more than one year), but such rotational forces will also produce Coriolis acceleration. Currently, three approaches are being used or considered as continuing countermeasures to the deleterious effects of microgravity on the crew: physical exercise, lower body negative pressure (LBNP), and drug therapy. There is still little knowledge on the relative merits of these conditioning efforts and none, of course address the similar problems which would afflict other organisms which might be components of a biological life support system.

A.4.2 Earth gravity and microgravity simulation

Bed rest studies have often been employed as a substitute to study the effects of simulated weightlessness (e.g. Chobanian *et al.* 1974; Greenleaf & Kozlowski 1982; Greenleaf 1983; Dallman *et al*. 1984; Pequignot *et al*. 1985; Annat *et al*. 1986; Martin *et al*. 1986; Beckett *et al*. 1986; Blamick *et al*. 1988; Jansson *et al*. 1988; Shangraw *et al*. 1988; Leblanc *et al*. 1990; Stuart *et al*. 1990; Fortney *et al*. 1991; van der Wiel *et al*. 1991; Pannier *et al*. 1991; Arnaud *et al*. 1992; Greenleaf *et al*. 1992; Palle *et al*. 1992; Frey *et al*. 1993; Kawai *et al*. 1993; Lacolley *et al*. 1993; Hartikainen *et al*. 1993; Melchior & Fortney 1993; Vernikos *et al*. 1993).

Early bedrest and LBNP studies showed that oral rehydration provided a large increase in plasma volume and a reduction in stressed heart rate response (stress by LBNP or gravity), but only for a period of a few hours. These results developed into the simple procedure of oral rehydration shortly before re-entry. Some of the more recent results from bed rest studies have been mentioned in the previous chapter; others were, for example:

(i) An early (several days) increment in resorption parameters, like fasting urinary calcium/creatinine and hydroxyproline/creatinine ratios, or serum calcium and phosphate levels, and a decrease in serum 1,25-dihydroxyvitamin D (van der Wiel *et al*. 1991).

(ii) decreased whole-body protein synthesis with low dietary protein (Stuart *et al*. 1990); indication of the involvement of other factors than leg compliance, as altered cardiac or autonomic nervous function, or blood volume, to orthostatic intolerance after bed rest (Melchior, Fortney 1993).

(iii) an increased LBNP-induced sympathetic activity with regard to deficient vascular resistance after 1 day of head-down bed rest (Lacolley e*t al*. 1993).

(iv) benefits of isotonic exercise for the maintenance of plasma volume (Greenleaf *et al.* 1992).

(v) no change in (Frey *et al.* 1993), or an (early) increase (Kawai *et al.* 1993) in cerebral blood flow with head-down tilt bed rest.

Ingestion of isotonic salt solution combined with LBNP proved successful in returning plasma volume to baseline during extended bed rest (Hyatt & West 1977). Reduced chronotropic response and increased plasma volume with orthostatic challenge has also been observed with several hours of hydration plus LBNP (Fortney 1991). A recent investigation has shown that saline ingestion during pseudo-orthostatic challenge (1 l isotonic NaCl, 30 mm Hg LBNP) reduces plasma volume loss (from 14 to 6%) and less than 70% of the ingested 'extra' volume went into the extravascular space of the lower body (**Aratow *et al.* 1993**).

Artificial gravity may solve most of the problems caused by weightlessness, but within a rotating environment, stimulations of the vestibular, visual, and proprioceptor systems may produce symptoms of vertigo, disorientation, lassitude, postural aberrations, or nausea (motion sickness). Test subjects of the Pensacola Slow Rotation Room responded with a deterioration in well-being, frequently associated with a feeling of increasing lethargy. Adaptation and subsequent tolerance has been shown to occur for rotation speeds up to 10 rpm; no symptoms developed at 1 rpm or less (Green *et al.* 1971).

The effects of rotation on walking abilities on circular and flat walls was studied at the Langley Rotating Space Station Simulator, a circular platform of 12 m diameter with a 1.8 m vertical wall at the periphery. Subjects were suspended horizontally by a servomechanized boom and allowed to walk on the walls. Rotation speeds varied from 3 to 10.5 rpm, corresponding to gravitational accelerations from $0.05 \times g$ to $0.75 \times g$ at the level of the subject's feet. At $0.05 \times g$, while it was possible to walk in the direction of rotation, the test persons were unable to walk against the direction of rotation. Walking in the direction of rotation at a level between 0.17 and $0.3 \times g$ was most comfortable. At higher gravitational accelerations, the subjects reported sensations of leg and body heaviness which were disturbing above $0.5 \times g$ (Letko & Spady 1968).

For a rotating station of more than 20 m radius, the gravity gradient is not critical; motion sickness and the deviation of limbs during movement pose the major problems. Even subjects highly susceptible to vestibular side effects are not handicapped on sudden exposure to 1 rpm; some individuals adapt and function in environments rotating at rates as high as 10 rpm, but the ability to adapt varies from person to person, taking as long as 2 weeks to occur (Graybiel 1975). The only adaptation schedule which was able to provide a symptom-free adaptation to 10 rpm was a 9-step increase over a period of 25 days (Graybiel 1968). These findings suggest that the time required to effect adaptation can be greatly shortened by setting up an adaptation schedule for controlled head movements and stepwise increases in angular velocity.

A.4.3 In flight experimental evidence

LBNP (up to -50 mm Hg) was used extensively during Skylab missions to stress the cardiovascular system, to determine the extent and time course of deconditioning, and to determine whether in flight data on LBNP would be useful in predicting post flight orthostatic intolerance. In the Soviet programme, typical usage of the LBNP (up to -65 mm Hg in the 'Chibis' suit) was one 20 minute session every 4 days, and for 50 min d^{-1} during the final two days of the mission (Gazenko *et al.* 1980).

The treadmill is a device which places high loads on leg muscles by using bungee cords to simulate gravity pulling the individuals towards the walking surface. All Soviet space stations provided treadmills. Rapid (about 10 min) fatigue precludes use of the treadmill as an aerobic exercise device. However, Skylab treadmill proved to be a suited countermeasure to muscular deconditioning (Nicogossian *et al.* 1989).

Ergometers for use in flight are bicycle-like devices which can be pedalled with the hands or feet. Heart rate and metabolic level can be studied as a function of generated power ($W h^{-1}$). The ergometer used on Skylab operates within the range of 25 to 300 W, and at 20 to 80 rpm. The bicycle ergometer does not generate force levels approaching those used during ambulation. It is not thought to be an effective musculoskeletal or cardiovascular countermeasure on its own (Rambaut 1988).

Antigravity suits are standard equipment for fighter pilots. They are also used aboard the Space Shuttle during return to Earth to overcome symptoms of cardiovascular deconditioning (orthostatic intolerance). Standard antigravity suits work by inflating bladders in the leg and abdominal regions; the suit provides an increase in peripheral resistance, prevents fluid loss into the tissue spaces, increases venous return, and supports the diaphragm and thus the heart. The antigravity suit improves venous return and postflight orthostatic stability (Gazenko *et al.* 1981).

The penguin suit which is worn about 16 h during the normal day's activities, including exercise, provides passive stress on antigravity muscles (Rambaut 1988). The design of the suit is such that the cosmonaut can adjust the degree of tension in the elastic elements in order to stress specific muscle groups.

Studies conducted during the early Shuttle flights on a total of 26 trials (17 subjects utilizing the saline countermeasure and nine subject not using any countermeasure) demonstrated protection from orthostatic intolerance during post flight Stand Tests 1 to 2 h after landing (Bungo *et al.* 1985). A tendency toward reduced efficiency of salt water loading was noted in the Shuttle programme (Nicogossian 1989; Nicogossian *et al*. 1994).

A.4.4 Conclusions

Careful selection of countermeasures alone or in combination, and definition of timing, is needed to optimize the readaptation capacity of astronauts upon return to 1 × g (or landing on other celestial bodies after long term flights). The potential of possible side effects is not yet clear. Sufficient scientific information can only be derived from well-designed investigations in flight with a suitably large number of single observations. Because there are many potential variables in each mission, erroneous conclusions can be drawn by combining data from dissimilar test situations, and standardisation of conditions needs to be more firmly applied to future investigations.

Two of the host of major questions surrounding the concept of artificial gravity are: how much artificial gravity is needed? and what are the physiological limits to radius and angular velocity? Advantages of large scale rotating habitats, in addition to the medical ones, include ease of fluid and waste management, simpler designs of ecological life support systems, and potential benefits to crew psychological well being and social interactions. The decision to build large rotating habitats of significant complexity and cost might be reasonable if it is shown that the pathological effects of long term space habitation are not preventable through other less expensive means. Economically, there will be a trade-off between the development & construction costs of the habitat versus the rehabilitation costs associated with the recovery of crews following long duration flights. Rationalizing the development of a rotating space habitat is based significantly on the assumption that artificial gravity can stress and recondition the various systems of the body.

Rotating habitats may be necessary even in facilities on planetary surfaces such as Mars or the Moon, where reduced gravity levels may lead to physiological deconditioning similar to that experienced in microgravity. The maximum angular velocity to which an average group of individuals might be able to adapt, the time-scale of adaptation, and the degree of discomfort involved are all completely unknown. We also have yet to establish the minimum gravitational acceleration which is required to maintain normal physiological functioning of each of the major systems in the human body, and the exposure time which must be spent at that acceleration to ensure normal functioning. Proposed facilities to investigate physiological effects of artificial gravity include the following.

(i) The artificial gravity sleeper (AGS) concept, which is a 2 m radius centrifuge to rotate a subject about an axis near the head, providing a centripetal acceleration gradient which is maximal in the feet and near zero at the head. It has been hypothesized that rotation during sleep will help reverse fluid shifts, maintain vascular tone, and provide acceleration for musculoskeletal system, lymphocytes, etc.

(ii) A fractional gravity facility, with a 1 × g jogging track, in which the crew would live, work, and sleep in 0.5 × g, but for 0.5 to 1 h during the day they would jog around a track in the direction of rotation of the habitat, in effect increasing their angular velocity and thereby providing additional gravitational stress.

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