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Review

Biological Effects of Space Radiation on Human Cells: History, Advances and Outcomes

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Space radiation/Low-dose/Heavy ions/Individual susceptibility.

Exposure to radiation is one of the main concerns for space exploration by humans. By focusing deliberately on the works performed on human cells, we endeavored to review, decade by decade, the technological developments and conceptual advances of space radiation biology. Despite considerable efforts, the cancer and the toxicity risks remain to be quantified: 1) the nature and the frequency of secondary heavy ions need to be better characterized in order to estimate their contribution to the dose and to the final biological response; 2) the diversity of radiation history of each astronaut and the impact of individual susceptibility make very difficult any epidemiological analysis for estimating hazards specifically due to space radiation exposure. 3) Cytogenetic data undoubtedly revealed that space radiation exposure produce significant damage in cells. However, our knowledge of the basic mechanisms specific to low-dose, to repeated doses and to adaptive response is still poor. The application of new radiobiological techniques, like immunofluorescence, and the use of human tissue models different from blood, like skin fibroblasts, may help in clarifying all the above items.

INTRODUCTION

Exposure to space radiation is one of the main concerns for space exploration by humans.[1–4] However, the sources of space radiation, their composition, their flux, their dose and their biological effects were unknown fifty years ago and are not yet fully characterized. While recent advances in radiation biology permit to establish the first milestones of the radioprotection rules for spacecrafts crews,[5,6] a number of questions about the biological effects of space radiation remain unresolved. This is notably the case for the quantification of the risk of radiation-induced cancers, the understanding of some phenomena occurring specifically after radiation low-dose, the relative contribution of heavy ions in the response to space radiation, and the influence of the individual susceptibility in the final outcome.[7–10] The reply to all these questions requires precise measurements of the radiation dose itself and relevant choices of biological endpoints and models. We attempted here to describe, decade by decade, the historical evolution of concepts, techniques and data interpretation, by focusing deliberately on human cells data (Fig. 1).

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HISTORICAL OVERVIEW OF SPACE RADIATION

Ancillary results and theories until the 1960's

The discovery of cosmic rays. The potential hazards of manned space travels and the habitability of cosmos were considered very early. One of the most famous examples is that of Kepler who warned that extraterrestrial trips would require *ships fit to withstand the breezes of heaven*.[11] Scientists became aware that ionizing space radiation generate from three different origins: cosmos, sun and Earth. Advances in each area were unequal and naturally followed the basic and technological developments of physics specific to each item. Hence, the early developments of electroscopes illustrated by the pioneer works of Pierre Curie made possible the assessment of microcurrents due to particles crossing the atmosphere. In 1910, the Italian physicist Pacini suggested that the background noise measured by electroscopes is due to the Earth ground.[12] Wulf, a Jesuit priest, demonstrated that half of the radiation emitted by the Earth ground disappears at the top of the Eiffel tower. Thank to balloon experiments, Hess observed that the ionization density of atmosphere progressively decreases up to 1000 m, but increases above 1800 m, suggesting an impact of extraterrestrial phenomena. In 1912, Hess showed that this increase is independent of the hour of the observation and remains

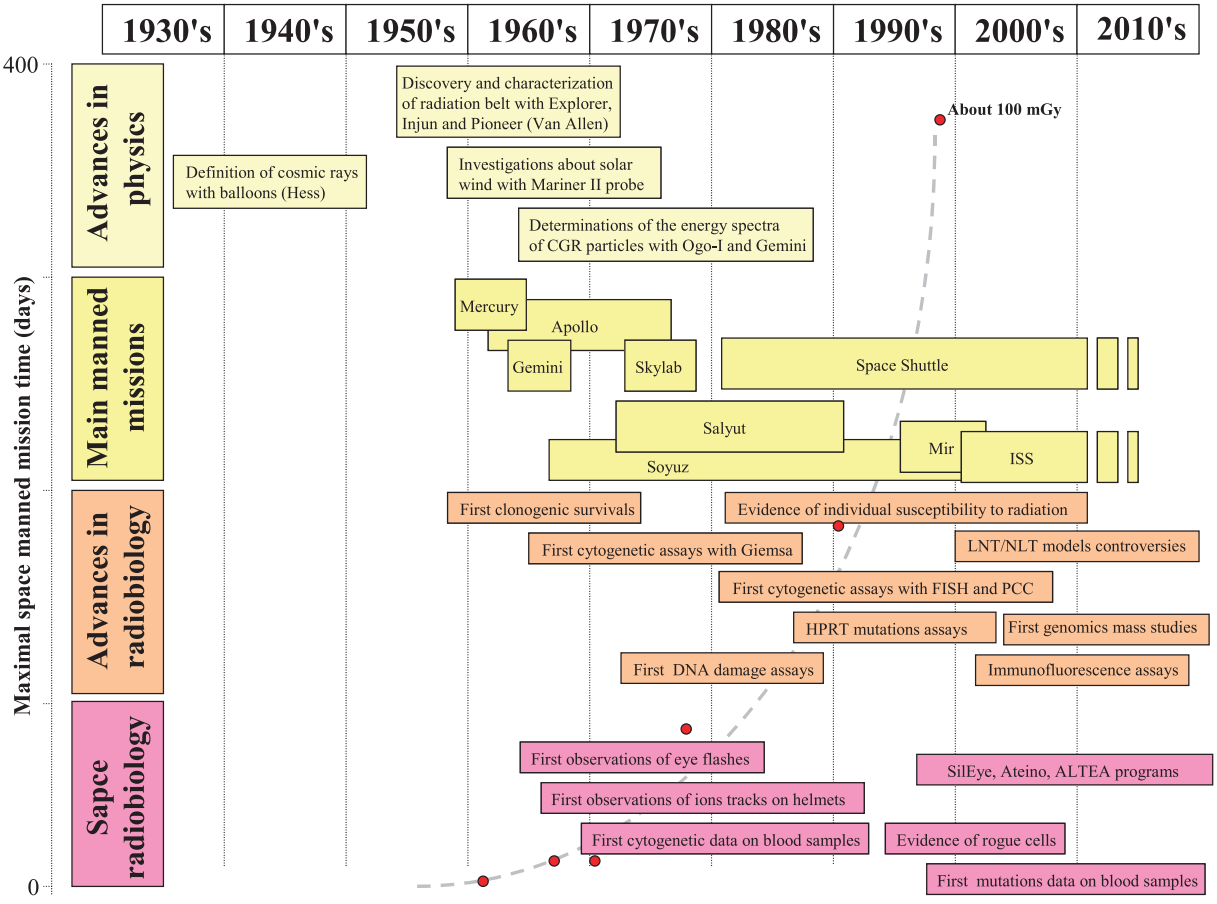

Fig. 1. Synoptic table of advances in space radiation biology. Grey dashed line illustrates the continuous increase of space mission duration with the maximal values for Mercury, Gemini, Apollo, Salyut, Skylab and Mir.

unchanged during a solar eclipse. Although the term "cosmic rays" was used lately by Millikan,[13] it was the first demonstration of the existence of radiation generated by cosmos, different from the natural radioactivity emitted from the Earth ground. In 1936, Hess received the Nobel Prize for his discoveries.[14] In parallel to the Hess's works, the technological developments of particles counters permitted to conclude that, in addition to γ-rays of more than 40 MeV, the primary cosmic rays consist in very energetic (10^8–10^{20} eV) protons and nuclear particles of atomic numbers up to 40[15–17] (Fig. 2). Lastly, between 1930 and 1940, the observations that there is an abnormally high relative abundance of the iron-associated elements in the galactic component radiation (GCR) suggested the nucleosynthesis origin of cosmic rays.[12] During all these decades, numerous questions raised about the biological impact of cosmic rays. However, the techniques for investigating biological effects were not available at this period and the biological endpoints were not yet defined to perform further investigations.[12]

The discovery of the Van Allen radiation belt. Space radiation research of the 1950's did mainly focus on magnetism. The works of the pioneers like Gilbert, Gauss or Poincaré suggested that charged particles may be influenced by the Earth's magnetism and that a ring current exists around the Earth. However, the experimental proofs of such phenomenon were confronted to technological limits: better than balloons, artificial satellites were required to perform large-scale measurements. Explorer I satellite and thereafter Pioneer IV allowed Van Allen and Franck to point out the existence of the Earth's radiation belt in 1958.[18,19] They revealed that this belt acts as a shield against charged particles. At the beginning of the 1960's, protons and electrons were found to be the major constituents of the Van Allen belt (Fig. 2 and 3). While it appeared that protons and electrons may be produced by solar UV- and X-rays-induced dissociation of hydrogen atoms, the most convincing theory supported that they are likely generated by decay of secondary neutrons emitted from cosmic charged particles.[20] The nuclear tests proceeded by USA and Soviet Union in high atmosphere between 1958 and 1962 added a significant number of trapped electrons between 1.2 and 2.0 Earth radii, and created some discontinuities in the natural particle distribution of the Van Allen belt.

First investigations about solar radiations. In the same

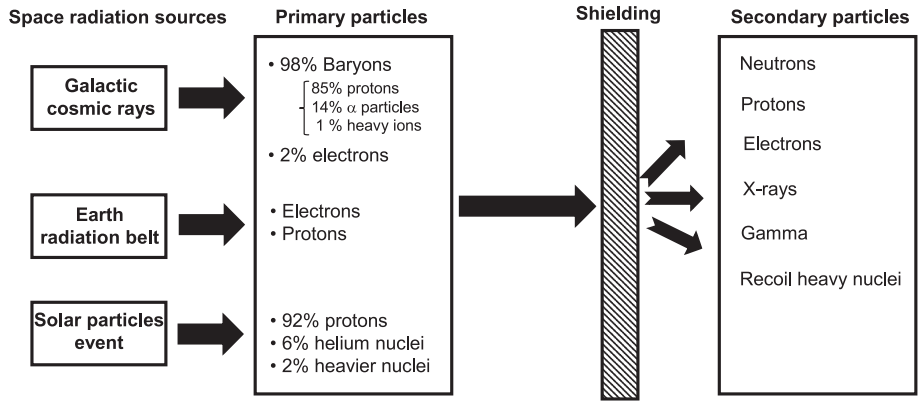

Fig. 2. Recapitulative scheme of the three sources of space radiation and their secondary particles.

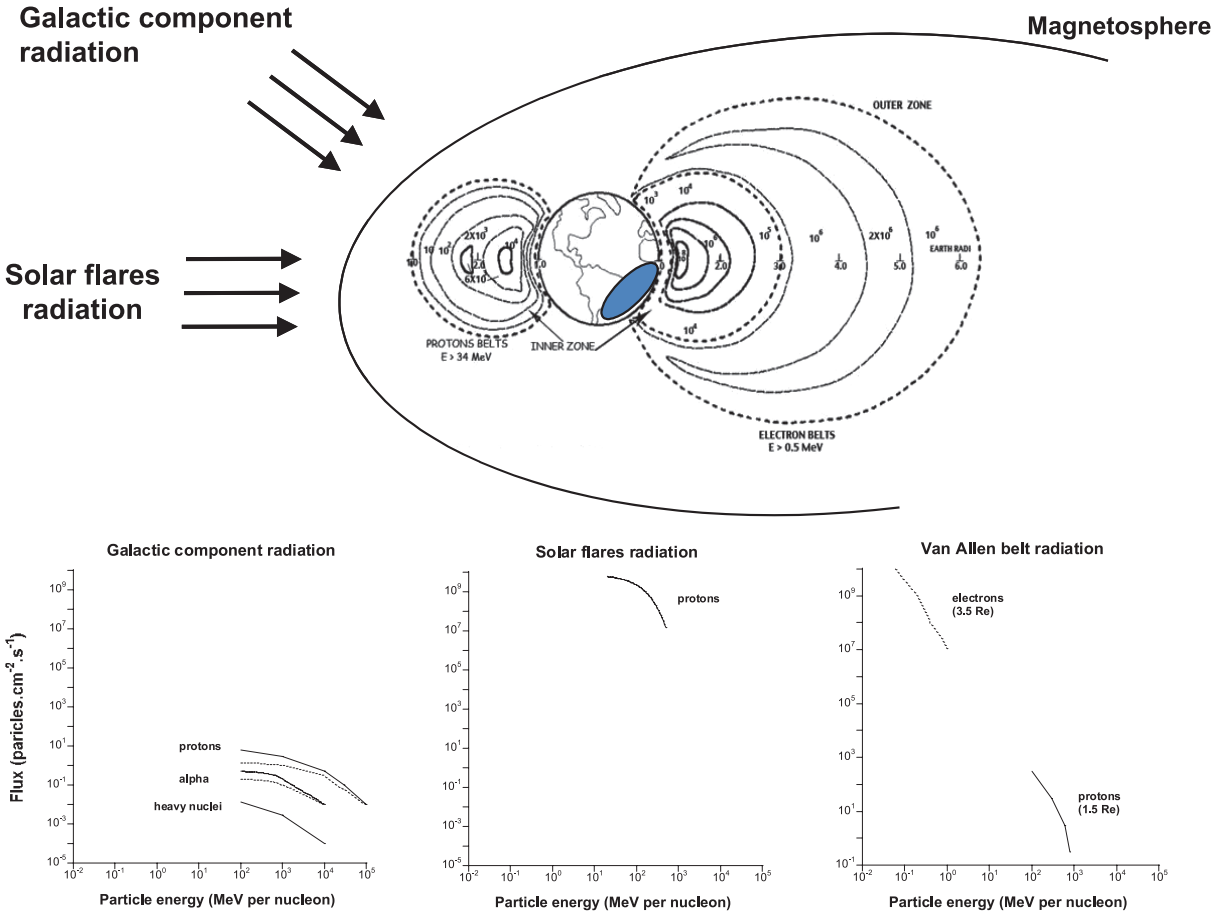

Fig. 3. The three sources of space radiation and the Van Allen belt (from[12]). The South Atlantic Anomaly is represented by a hatched area. In the lower panel, particles flux data from[16] are summarized and plotted against energy in the same scales. For the GCR data, dashed lines indicate the values obtained during solar flares. For the Van Allen belt radiation, Re represents Earth radius.

decade, data from space probes missions like Mariner II provided interesting data about the solar wind. Neugebauer and Snyder (1964) found that protons are the most frequent particles emitted from the sun: about 3 protons per cm^3 at about 600 $km.s^{-1}$ were detected close to the Earth during a period of solar quiescence.[21] The velocity of these particles was suggested to increase 10 to 100 times during solar activity. Until the 1950's, only ionizing chambers were used for determining of the energy spectrum of solar wind particles[22] (Fig. 2).

Brief summary of space radiation research until the 1960's. The three sources of space radiation have been pointed out in the first half of the XXst century (Fig. 1). The predominance of protons for each of these sources was demonstrated at the end of this period (Fig. 2). The biological hazards of space radiation were evoked early but scientists were unable to provide quantitative estimation of the risks, not only because radiobiology techniques were not sufficiently developed but also because molecular and cellular endpoints reflecting biological risks were not known or consensual[23] (Fig. 1).

The big steps of the 1960's

First determinations of the energy spectra of the cosmic rays. Thank to the technological advances and the Cold War, the essential of the energy spectra of each source of space radiation can be considered as essentially resolved in the 1960's. The GCR particles were found to consist in 98% baryons and 2% electrons. The baryonic component is composed of 85% protons, 14% alpha particles and about 1% heavy elements such as iron[16] (Figs. 1 and 2). The flux of these cosmic particles emitted isotropically is however low (about 4, 0.4 and from 10^{-4} to 10^{-2} particles.cm^{-2}.s^{-1} for protons, alpha particles and heavier ions, respectively).[16] Their energy may range from 10^{-3} to 10^{11} MeV (Figs. 2 and 3). It is noteworthy that solar activity was shown to affect the lower-energy portions of each particle distribution. Between 1964 and 1965, data accumulated by the Ogo-I satellite demonstrated that the C-N-O, the Li-Be-B and the Fe groups represent about 0.83%, 0.23% and less than 0.05% of the GCR particles flux, respectively.[24]

Characterization of the Van Allen belt. Thank to the Injun and Explorer missions, the inner zone of the Van Allen belt was shown to consist in a majority of protons and a minority of electrons (Figs. 2 and 3). This distribution is inversed in the outer zone. The inner zone consists in protons that range from 10^{-3} to 10^{2} MeV with flux from 1 to 10^{5} particles.cm^{-2}.s^{-1}. The maximal flux is observed at about 1.5 Earth radii (about 3200 km altitude). At 500 km, the flux is about 10^{2} protons cm^{-2}.s^{-1}. The outer zone is larger than the inner zone and reaches up to 10 Earth radii. The energy of protons is lower (from 0.1 to 5 MeV on average) but their flux is higher (from 10^{6} to 10^{8} protons cm^{-2}.s^{-1}). At 500 km, the flux of protons of the outer zone may however be neglected (Fig. 3). Trapped electrons of the inner zone reach flux of 10^{4} particles.cm^{-2}.s^{-1} from 1.4 to 2.4 Earth radii at energies ranging from 10^{-1} to 10^{1} MeV. In the outer zone, similarly to protons, the flux of electrons is higher (up to 10^{8} electrons.cm^{-2}.s^{-1} at 5 Earth radii) and their energies are lower (from 10^{-3} to 10^{0} MeV). At 500 km, the electron component of the inner zone is very low whereas it reaches 10^{8} particles.cm^{-2}.s^{-1} with energy higher than 40 keV in the outer zone[16] (Fig. 3).

The South Atlantic Anomaly. The South Atlantic Anomaly (SAA) is the area where the inner Van Allen radiation belt is the closest to the Earth surface, leading to an impressive flux of protons and electrons (more than 100 protons cm^{-2}.s^{-1} at 500 km). The radiation threshold altitude varies from 200 km over the eastern coasts of Brazil and Argentina to the tip of South Africa and to 1400 km over certain parts of the northern hemisphere (Fig. 3). The existence of SAA was suggested in 1950's but identified only at the end of the 1960's. The scientific community became aware that SAA raises actual technological questions for satellites (radiation-induced injuries on electronics and materials) and overall health problems. To date, the Hubble Space telescope does not take observations while passing the SAA. While space stations cross SAA about 15 times a day for 15 min, we will see in the next chapters that these chronic exposures raise some important radiobiological questions.

Characterization of the solar particles events. In the same period, although energy spectrum may vary drastically from event to event, particles from solar wind and solar flares were characterized. The solar wind is a high flux (10^{8} particles.cm^{-2}.s^{-1}) of some keV protons. The solar flares generally consist in 92% protons, 6% helium nuclei and less than 2% heavier nuclei at some MeV.[16] The solar particle events (SPE) are relatively rare and occur most often during the solar maximum phase of the 11-year solar cycle. During these periods, the interplanetary magnetic field generated by the sun provides some protection to the inner solar system, decreasing the GCR intensity.[25]

Pioneer calculations for shielding. The development of theories of interaction between radiation and matter significantly contributed to the calculations for radiation shielding for the first manned spatial missions. This was notably the case with the definition of the stopping power that Bethe proposed in the 1950's.[26] About 37% of high-energy protons can penetrate 100 g.cm^{-2} materials without nuclear interaction. Incident protons from GCR, Van Allen radiation belt and sun remain therefore of biological interest. The great majority of protons can however loose their energy by providing secondary protons and neutrons emitted in the forward direction with a larger fraction of incident energy on one hand and electrons, γ- and X-rays emitted isotropically at low energy on the other hand.[27] With regard to electrons, the photoelectric effect, Compton scattering and pair-production phenomena were sufficiently known at this period to permit first calculations for shielding.[16] Conversely, the interactions of heavier nuclei with matter were still misknown but scientists were already aware that high-Z shielding drastically increases the emission of secondary particles after impacts of heavy ions.[16,28,29] This is notably the case for secondary neutrons due to nuclear interactions. Hence, aluminium appeared to be a good compromise for low Earth orbitals but not for long travels throughout the cosmos. Numerous active and passive strategies for shielding are now proposed. For example, new materials like Kevlar may provide satisfying shielding against heavy

ions.[30,31] However, the nuclear reactions, and notably the photoactivation reactions induced by high-energy particles from space remain misknown. We will see in the next chapter that such field may be however important to understand the space radiation response.

First estimations of radiation dose in space. An important conclusion from data obtained in the 1960's was that protons represent the most important contribution to the dose.[32] However, dose and dose rate are also important parameters to better predict the radiation response. Average dose rate in OVI satellites was early estimated to be at the order of 1 $\mu Gy.min^{-1}$ in low Earth orbit. Solar activity can multiply this dose by 5 to 30. With regard to the GCR particles, the average dose rate assessed by Gemini IV, VI and VII missions ranged between 0.01 to 1 $\mu Gy.min^{-1}$.[32,33] The reviews of Curtis and that of Benton and Benton provided an impressive survey of doses received during all the manned missions.[25,32] The Table 1 summarizes the radiation exposure on Mercury and Gemini missions assessed with passive dosimeters. After the Gemini missions, the Apollo program included a systematic assessment of radiation dose by personal and passive dosimeters in addition to spectrometers (Table 2). Dose rates calculated on the basis of the total mission time were relatively low, ranging from 0.1 to 0.9 $\mu Gy.min^{-1}$, in good agreement with the exposure of Gemini missions (Fig. 4). A dose rate of 0.17 $\mu Gy.min^{-1}$, corresponding to 245 µGy per mission day can be considered as a good approximation relevant for all the Gemini and Apollo missions (Fig. 4). Dose measurements in Vostok, Voskhod and Soyuz reached similar values[25,32] (Table 3). When dose is plotted against mission time, a correlation appears: the total doses received by Apollo crews were logically found larger than for Gemini missions (Fig. 4). This relationship between time and dose remains the same even by considering total mission time *excluding circumlunar and lunar surface times*. This last conclusion suggests that the GCR contribution is low with such missions durations. Even if there is no enough data to conclude that GCR contribution increases also with time of exposure, it is noteworthy that the Apollo X, XII, XVI, XVII received two-fold higher dose than expected (Fig. 4). Although no SPE was officially registered for these missions, it was said that "*one small event was detected by a radiation sensor outside the Apollo 12 spacecraft, but no increase in radiation dose to the crewmen inside the spacecraft was detected*".[34] Conversely, it is known and verified that Apollo XIV crossed intense solar flares explaining the impressive 11.4 mGy measured during the mission while a 5 times lower dose was expected. This last dose was the highest radiation dose of all the manned spatial missions at this period.[25,32]

First radiobiological investigations. The 1960's period corresponds also to the development of cytogenetics and clonogenic cell survival assays that permitted considerable advances in radiobiology (Fig. 1). First microscopic observations showed that ionizing radiation cause non-reparable chromosome fragments that can lead to micronucleus formation or apoptosis.[35,36] Logically, space radiation research followed such developments with a number of experiments on the Biosatellite and Gemini missions. However, these experiments involved a number of different animal and vegetal models. Furthermore, most of the studies aimed to investigate the effect of microgravity rather than the radia-

Table 1. Summary of the total dose and dose rate received during Mercury and Gemini missions (from[25,32])

Missions and Year	Exposure time (day)	Total dose (mGy)	Average dose rate ($\mu Gy.min^{-1}$)
Mercury MA-8 (1962)	0.38	0.05–0.6	0.1–1
Mercury MA-9 (1962)	1.43	0.12–0.182	0.06–0.9
Gemini III (1965)	0.2	0.20–0.45	0.7–1
Gemini IV (1965)	4.04	0.45–0.70	0.07–0.1
Gemini V (1965)	7.96	1.72–1.95	0.15–0.17
Gemini VI (1965)	1.08	0.22–0.31	0.14–2
Gemini VII (1965)	13.77	1.05–2.31	0.053–0.12
Gemini VIII (1966)	0.45	0.1	0.15
Gemini IX (1966)	3.04	0.14–0.27	0.032–0.06
Gemini X (1966)	2.95	6.18–7.79	1.45–1.82
Gemini XI (1966)	2.97	0.23–0.39	0.05–0.08
Gemini XII (1966)	3.98	0.20	0.035
Summary	**Up to about 13 days**	**Up to about 2 mGy on average (nearly 8 mGy for Gemini X)**	**0.03 to 2 $\mu Gy.min^{-1}$**

Table 2. Summary of the total dose and dose rate received during Apollo missions (from[25,32])

Missions and Year	Exposure time (day)	Total dose (mGy)	Average dose rate (μGy.min^{-1})
Apollo VII (1968)	10.8 earth orbital	1.48–1.71	0.095–0.11
Apollo VIII (1968)	6.12 incl. 0.83 circumlunar	1.33–1.77	0.15–0.2
Apollo IX (1969)	10.04 earth orbital	1.82–2.39	0.12–0.16
Apollo X (1969)	8 incl. 2.56 circumlunar	3.86–5.60	0.3–0.48
Apollo XI (1969)	8.12 incl. 2.47 circumlunar and 0.9 lunar surface stay-time	1.65–1.88	0.14–0.16
Apollo XII (1969)	10.16 incl. 3.7 circum lunar and 1.3 lunar surface stay-time	5.80	0.4
Apollo XIII (1970)	5.95	2.40	0.27
Apollo XIV (1971)	9 incl. 2.79 circumlunar and 1.4 lunar surface stay-time	11.40	0.87
Apollo XV (1971)	12.7 incl. 2.78 lunar surface stay-time	3.0	0.16
Apollo XVI (1972)	11.08 incl. 2.96 lunar surface stay-time	5.10	0.32
Apollo XVII (1972)	12.54 incl. 3.12 lunar surface stay-time	5.50	0.3
Summary	**Up to about 12 days**	**Up to about 5 mGy on average (about 11 mGy for Apollo XIV)**	**0.1 to 0.9 μGy.min^{-1}**

Table 3. Summary of the mean total dose and mean dose rate received during Salyut, Skylab, Mir missions (from[25])

Missions and Year	Exposure time (day)	Total dose (mGy)	Average dose rate (μGy.min^{-1})
Vostok (1960–1963)			0.05–0.31
Voskhod (1964–1966)			0.08–0.45
Soyuz 3- 9 (1968–1969)			0.09–0.14
Salyut (1974–1981)	16–185	1–23	0.07–0.125
Skylab (1973–1979)	28–90	16–77	0.08–0.6
Mir 01-23 (1986–1997)	126–366	22.4–92.9	0.11–0.35
Summary	**16–366**	**1–92.9**	0.05–0.6

tion effect itself. The experiments of Bender *et al*. performed with Gemini III and XI in 1967 and 1968 on human leucocytes irradiated by β particles from ^{32}P sources did not permit to conclude to any significant effect of space radiation[37,38] (Table 4). Soviets applied similar approaches with Cosmos-110 Biosatellite and Vostok missions but with the same technological and statistical limitations.[39,40] With regard to Apollo crews, despite a tendency of an increase in post-flight chromosome breaks, most of the cell cultures did not provide sufficient mitoses to be analyzed. Consequently, a general care must be taken about the interpretation of all the available data.[41] Chromosome breaks yields were however found two-fold higher in Apollo than in Gemini astronauts, suggesting for the first time a link between dose and the duration of the flight[41] (Table 4). Interestingly, despite some inter-individual variations, the yields of chromosome breaks in lymphocytes from astronauts who had no previous flight experience appeared generally lower than those from experienced astronauts. Lastly, post-flight aberrations were considered to be about two-fold higher than pre-flight values[41] (Fig. 1).

The first description of eye flashes. Another aspect of biological consequences of space radiation exposure on human cells was the subjective sensations of lights on eyes, commonly called *eye flashes*. Eye flashes were first observed by Apollo crews (Fig. 1). Such phenomena were initially thought to be due to μ mesons from statistical arguments. However, the actual origin of the eye flashes remained still unresolved at the end of the 1960's.[12] Primary or secondary neutrons and possibly heavy ions, rather than mesons were thereafter suspected to cause eye flashes. Observations on helmets of Apollo astronauts revealing numerous tracks of metallic ions as heavy as zinc and nickel (up to 10^{11} track.cm^{-2}) were questionable since these heavy ions are

Table 4. Summary of the cytogenetic data obtained from the indicated space manned missions

Reference	Mission(s)	Technique(s)	Endpoint(s)	Biological materials	Mission duration (days)	Estimated during the mission dose (mGy)	Conclusions
Bender *et al.*, 1967[38]	Gemini III	Giemsa	single- and multiple-break chromosome aberrations	Leucocytes of two ground-based donors irradiated during flight by β particles from ^{32}P sources	0.2	0.2–0.45	No increase of multi-break aberrations but **increase of single-break aberrations after the flight.** However blood samples from the flight crew show that the spaceflight itself did not induce aberrations.
Bender *et al.*, 1968[37]	Gemini XI	Giemsa	single- and multiple-break chromosome aberrations	id.	2.97	0.23–0.39	No increase of single- and multi-break aberrations s after the flight.
Gooch and Berry, 1969[116]	Gemini	Giemsa	single- and multiple-break chromosome aberrations	17 astronauts blood samples	nd. (0.2–13.77)	nd. (0.1–7.79)	**The majority of samples show 2-fold increase of chromosome breaks after the flight.**
Kimzey *et al.*, 1975[41]	Apollo	Giemsa	single- and multiple-break chromosome aberrations	Blood samples from Apollo astronauts	nd. (5.95–12.54)	nd. (1.33–11.4)	Technical problems: notenough mitoses in general. However, **the majority of samples show 2-fold increase of chromosome breaks.** There is a rather constant postflight aberrations yields which seems to be dependent on the duration of flight. **Breaks and aberrations values in experienced astronauts appear to be higher than the other crewmen.**
Lockhart, 1977[44]	Skylab	Giemsa	single- and multiple-break chromosome aberrations	Blood samples from 3 Apollo astronauts	Skylab 2 (29) Skylab 3 (59)	Nd Skylab 3 (38.4)	It is the description of the Skylab M111 experiment. Individual variations and no evidence of the flight itself is a significant factor in either Skylab 2 or 3 missions in contributing to increase chromosomal aberration yield. No difference between ground based crew members and astronauts but more aberrations by comparison with general population (influx of the use of radionucleides?).
Testard *et al.*, 1996[54]	Mir- Antares Mir- Altaïr	Giemsa and R-banding	Breaks, dicentrics, centric and acentric rings	Blood samples from 7 cosmonauts	30–180	20–455 (c)	Detectability threshold at 20 mGy. From 6 months flights, a great heterogeneity between individual s is observed and **rogue cells are observed at higher frequency.**
Khaidakov *et al.*, 1997[52]	Salyut-Mir	HPRT	Hprt mutation Deletion frameshift, etc	Blood samples from 5 cosmonauts	7–365	4–127	No excess of deletions and no correlation between deletions and flight duration. **However, more splicing errors, frameshifts and complex mutation in cosmonauts than in general population.**
Yang *et al.*, 1997[117]	NASA -Mir-18	FISH and giemsa	Dicentrics, chromosome breaks and sister-chromatid exchanges	Blood samples from 2 cosmonauts	115	52	**The frequency of reciprocal exchanges and total chromosome aberrations are higher post-flight than pre-flight.** However, yield of sister-chromatid exchanges remains unchanged.
Obe *et al.*, 1997[55]	Mir92 EuroMir94 and 95	Giemsa	Dicentrics, rings and sister-chromatid exchanges	Blood samples from 7 cosmonauts	120–198	57–94 mGy Low-LET 4.0–6.7 mGy High-LET	**Chromosome-type** but not chromatid-type aberrations **were significantly elevated after space flights when compared to pre-flight values.** Some rogue cells were detected. In agreement with Testard *et al.*, (1996).
Druzhinin, 1999*[118]	Mir	Giemsa	Chromosome breaks and aberrations	nd	194 and 196	nd	No significant increase of the number of aberrant cells and the rate of chromosomal aberrations.

Table 4. Continued.

Reference	Mission(s)	Technique(s)	Endpoint(s)	Biological materials	Mission duration (days)	Estimated during the mission dose (mGy)	Conclusions
Fedorenko *et al.*, 2000* and 2001[119,120]	Mir	Giemsa	Chromosome breaks and aberrations	Blood samples from 36 cosmonauts	Up to 120–180	20–280	**The frequency of chromosomal-type aberrations significantly increases after flight, decreases during inter-flight of about 1.5–2 years without reaching the control levels**
George *et al.*, 2001[121]	NASA-Mir and "taxi" STS	FISH	Breaks, translocations, exchanges	Blood samples from 8 astronauts including 6 NASA-MIR and 2 "Taxi" astronauts	90–120 for NASA-Mir and 10 for "Taxi"	31–44	**Yield of chromosomal aberrations increases after long flights but not after " taxi". More complex-type aberrations after long flights. Presence of rogue cells.**
Wu *et al.*, 2001[122]	STS-103	FISH	Breaks, translocations, exchanges	Irradiation of blood samples from one astronaut after flight with ^{137}Cs source	8	16	Study about synergy between microgravity and radiation. No difference between pre and post-flight data.
George *et al.*, 2002, 2003, 2004[123,124]	Mir and ISS	FISH and PCC	Breaks, translocations, exchanges. Clonal aberrations before and different days after flight	Blood samples from 10–12 astronauts	90–120	nd.	**The frequency of simple translocations increases post-flight** but there are very few complex-type exchanges. The frequency of clonal aberration decreases after 100–240 days post-flights.
Greco *et al.*, 2003[125]	Mir and ISS	FISH and PCC	Breaks, translocations, exchanges	Blood samples from 1 Italian and 8 Russian cosmonauts. Additional irradiation with 6 MeV X-rays	9–312	nd.	Increase of chromosome aberrations after flight but no correlation with flight duration,, the number of flights or extra-vehicular activity. However, enhancement of radiosensitivity after flight after irradiating sample after 6 MeV gamma-rays.
Durante *et al.*, 2003[126]	Mir, ISS and STS	Giemsa and FISH	Dicentrics (Giemsa), translocations (FISH)	Blood samples from 33 cosmonauts including 15 involved in long flights and 8 in "taxi" flights	8–199 (cumulative : 8–748)	1.4–118 (cumulative : 2–289)	**Yield of dicentrics increases after long flights but not after "taxi"** For multiple flights, dicentrics and inter-chromosomal exchanges are not correlated with cumulated dose or time in space. Tendency of aberrations to decrease some 1000 days after flights.
George *et al.*, 2005[127]	Nd. Mir and ISS, and STS?	FISH	Chromosome exchanges before and different days after flight	Blood samples from 6 astronauts	20–200	nd.	**Yield of total exchanges increases immediately after -flight** but generally decrease 20–700 after flights.
Horstmann *et al.*, 2005[128]	ISS	Giemsa and FISH, mFISH	Dicentrics and exchanges	Blood samples from 11 astronauts including 7 involved in long flights and 4 in short-term flights	6–192	90 for long flights and 2 for short term flights	Yields of dicentrics increases post-flight only if data for all the astronauts are pooled. No inversions , no complex aberrations were detected.
Pelevina *et al.*, 2007*[129]	Mir and ISS ?	Comet and Giemsa	DNA breaks and chromosome aberrations	Blood samples from pilots and cosmonauts	Nd	Nd	Adaptive response was registrated in 3 cosmonauts.
Fedorenko *et al.*, 2008*[130]	Mir and ISS ?	Giemsa	Dicentrics and exchanges	Blood samples from 37 cosmonauts	Nd.	Up to 110 mGy	**Increase of stable and unstable aberrations. Frequency of dicentric and acentric rings depend on flight duration, cumulative dose and dose rate.** Frequency of chromosomal aberrations remains altered after several years after flight.
George *et al.*, 2010[131]	ISS	PCC and FISH	Exchanges	Blood samples from 37 cosmonauts	95–215		**Significant decrease of chromosome exchanges after the first 220 days post-flight despite large inter-individual differences.** Same tendency for dicentrics but no statistical singificance.

*Article in Russian – No detailed data available.

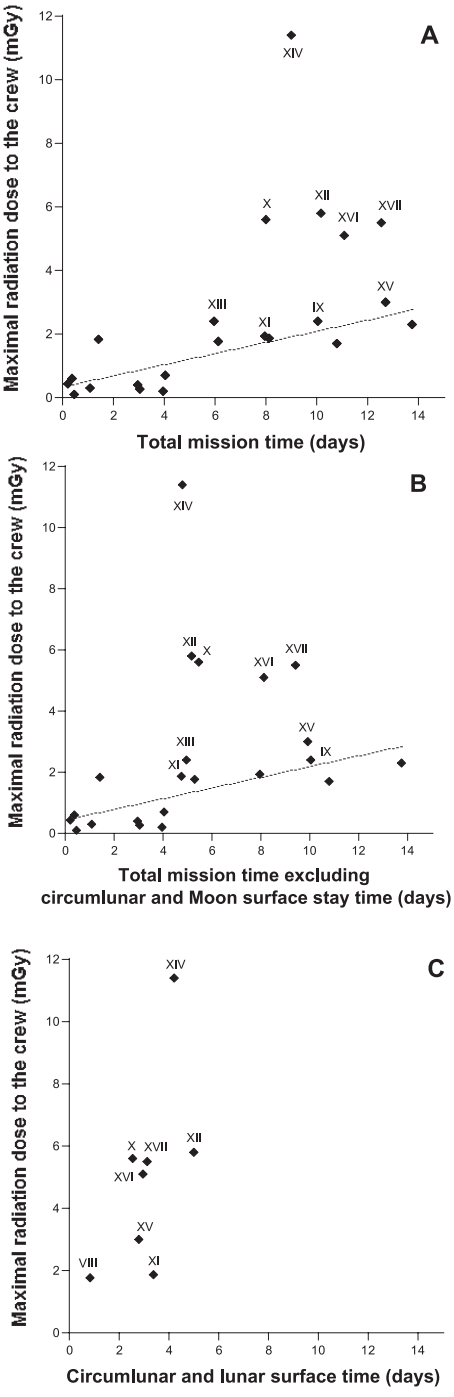

Fig. 4. Maximal total radiation dose to the Gemini and Apollo crews as a function of total mission time (a), of total mission time excluding circumlunar and lunar surface time (b) and of circumlunar and lunar surface time (c). Roman figures indicate the number of the Apollo mission. A linear regression formula has been applied to the data shown (dotted line): and was found to be y = 0.346 + 0.175x; r = 0.81 for panel a and y = 0.439 + 0.173x; r = 0.74 for panel b.

very rare in space. Such observations suggested therefore that the technical environment of spacecraft itself adds extra-complexity in the actual spectrum of secondary particles.[32] The existence of nuclear interaction between high-energy protons and electrons with the abundant metallic stuff surrounding crews may be evoked.

Brief summary of space radiation in the 1960's. Short-term (less than 15 days) missions performed in the 1960's at low Earth orbit received some mGy at an average dose rate of about 0.17 μGy.min^{-1} (245 μGy per mission day). However, this dose was not delivered continuously for three reasons, at least: inner and outer zones of Van Allen radiation belt deliver different spectra of protons and electrons corresponding to different secondary particles at different energies and dose rates; 2) passage above the SAA (about 15 times a day) results in a significant exposure that can increase the dose rate by a factor of 6[25]; 3) the unpredicted SPE may drastically increase the total dose. Cytogenetics observations revealed for the first time the major biological consequences of an exposure to space radiation: yield of chromosome breaks seemed to increase after flight but statistical significance was still needed. Data from eye flashes and helmets suggested the existence of a certain "hidden part" of heavy ions component, probably due to secondary particles generated by interaction of very high-energy particles with metallic stuff. The contribution of these heavy ions to the total dose of radiation remained unknown at the end of the 1960's.

Space radiation biology from 1960's to 2010's: from one space station to another

The Skylab space station. Skylab was the first US space station. Originated from the Apollo program stuff remains, it was launched in 1973. The Skylab space station permitted 3 manned missions (Slylab 2 to 4) before its disintegration in atmosphere in 1979. The major Skylab issues in the scope of this review are dosimetry, eye flashes and cytogenetics data.[42] The onboard dosimeters included passive and personal radiation dosimeters, and electron-proton spectrometer fixed outside the spacecraft. Skylab 2 and 3 missions received total doses and dose rates that were higher than the values of Apollo and Gemini missions (Tables 1–3) (Fig. 5). Particularly, the Skylab 4 (that was the first 90-day mission) received the impressive total dose of 77 mGy. This dose is 7 times higher than the highest values measured during Apollo XIV mission that was concerned by a SPE (77 vs. 11.4 mGy). However, Skylab 4 was 10 times longer than Apollo XIV (90 vs. 9 days) (Table 3).[25,34] Hence, the mean dose rates during these two missions are quite similar (850 vs. 1260 μGy per mission day). It is therefore noteworthy that, like Apollo XIV, Skylab missions were characterized by a dose rate higher than the general mean calculated from all the data shown in Fig. 4 (400 μGy per mission day) (Fig. 5). During the Skylab missions, the GCR component repre-

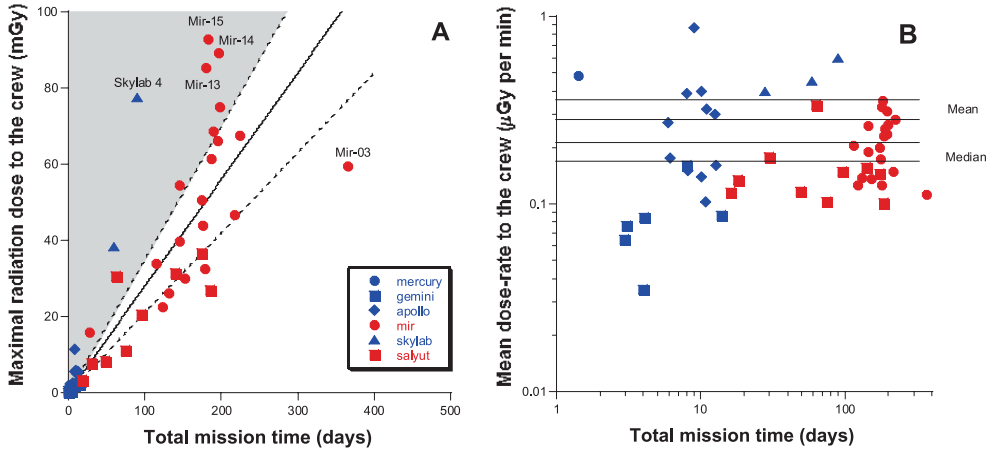

Fig. 5. Maximal total radiation dose (a) and mean dose rate (b) as a function of mission time received by all the manned space missions. In panel a, the grey zone indicates all the missions whose maximal total dose exceeds the mean + standard error (mean ± standard error are indicated in dotted line).

sented 20–30% of the crew mean doses, in agreement with theoretical calculations and measures. These remarks support therefore that: 1) the majority of the dose originates from protons of the Van Allen radiation belt and secondary particles generated by passage of the primary particles through spacecraft materials; 2) the contribution of GCR heavy ions to the dose is much lower than that of SPE. Since electrons from the Van Allen radiation belt were shown to be unable to penetrate into spacecrafts and since they do not have a significant impact during extravehicular activities, the electrons contribution to the dose originates mainly from secondary particles. Thermal and intermediate (0.02 eV to 2 keV) neutrons were found to contribute to the crew dose equivalent at a combined rate of approximately 1 μSv per day. Although the onboard technology available did not permit measurements of fast neutrons, they were calculated to contribute to 100 times more to the dose than the other types of neutrons.[34] Consequently, the unexpectedly high dose rates during Skylab missions were likely due to solar flares. From all the data available to date and accumulated in Fig. 5, the predictability of solar activities appears to be one of the major challenges for risk prevention.

During the skylab 3 mission, four French nuclear tests were proceeded in the Pacific area. Although radiation due to the tests were considered to be negligible, the eye-observation of the sites of detonation was considered to be potentially hazardous.[34] Besides, through Skylab missions and Apollo-Spyus Test Project (ASTP) (1975), a correlation between eye flashes and particles fluxes was pointed out, suggesting than particles of linear energy transfer (LET) greater than 5 keV.μm^{-1} may be required for eye flashes occurrence.[43] However, the impact of heavy ions still remained questionable since there is about one heavy ion per 1000 protons in the inner belt. Moreover, some other results seem to be contradictory. For example, a strong correlation was established between eyes flashes rate and passage through the SAA on Skylab whereas such correlation was not observed during Apollo missions.[41,42]

The so-called M111 experiments aimed to quantify chromosome breaks and aberrations before and after the flights, on the crew but also on the ground staff working with the crews. Surprisingly, despite of abnormally high dose rates, excess of chromosomal defects including chromatid breaks, chromosome breaks, deletions, and fragments were not detected in lymphocytes from Skylab 2 and 3 crews (Table 4). The flight itself was not considered as a significant damaging factor since no difference between ground staff controls and the crews was observed. In fact, structural rearrangements including dicentrics, exchanges, inversions, and translocations were found to occur more frequently in both groups than in studies of normal subjects, suggesting that radioactive sources and other hazardous stuff used during ground preparations of flights may impact on the yield of chromosomal defects.[44] However, like with the previous manned missions, whole data obtained from cytogenetic assays with Giemsa staining did not provide a sufficient body of data to definitively conclude on the importance of space radiation-induced hazards.

The Salyut space station. The Soviet Salyut represented a very ambitious space research program from 1971 to 1991 that led to both Mir space station and International Space Station (ISS) projects. It was composed of 7 Salyut, 3 Durable Orbital Station (DOS) and one Cosmos (Cosmos-557) vehicles. Launched in 1971, the Soviet Salyut I space station was, before Skylab, the first space station to orbit Earth. The studies performed with Salyut program in the scope of this review concern new radiation dosimetry data and some cytogenetics studies. However, there are very few published studies concerning Salyut specifically and no available data about eye flashes.

Performed with active and passive dosimeters and with different instruments, radiation dose in Salyut was assessed systematically and was fully documented.[45–49] The space radiation dosimetry was provided by thermoluminescent dosimeters and is summarized in Table 3 and Fig. 5. It is noteworthy that some technological advances have contributed to investigate high-energy neutrons (> 10 MeV) and gamma-rays (1–100 MeV).[49] The dose rate measured by crews was in the range of the other orbital missions and in agreement with the average dose rate of the previous manned missions (0.28 ± 0.07 μGy.min^{-1} ; about 400 μGy per mission day) (Table 3 and Fig. 5).

As evoked above, there were very few published cytogenetic data from blood samples of cosmonauts operating specifically on Salyut missions. One of the most interesting features of Salyut missions was that their duration progressively increased up to a maximum of 185 days for Salyut 6, while the longest mission in Skylab was 90 days (Table 4 and Fig. 1). The total dose received by Salyut 6 crews (27 mGy) was 2.85 times lower than that received by Skylab 4 (77 mGy) while the flight duration was 2 times longer.

In parallel with the Giemsa technique evoked above, the period 1971–1991 was marked by the use of the hypoxanthine phosphoribosyltransferase (HRPT) cloning assays. When radiation cause a mutation in the HPRT locus, cells cannot phosphorybosilate purine analog like 6-thioguanine and therefore survive in medium containing purine analog. The HPRT assay is an interesting tool to isolate mutated cells and identify induced mutations.[50] Hence, by applying this technique, abnormal significant biological endpoints were observed for the first time on lymphocytes from cosmonauts. Particularly, Khaidakov *et al.* (1997, 1999) did observed specific mutations spectra in blood samples of cosmonauts in Salyut and Mir who have completed spaceflights of 7 to 365 days. The doses received in space by these cosmonauts ranged from 4 to 127 mGy. HPRT mutant frequencies were found 2–5 higher than unexposed controls. Splicing errors, frameshifts and complex mutations were detected. The biological and clinical consequences of all these events are difficult to quantify, notably with regard to the risk of radiation-induced cancer. However they provide interesting clues about the potential long-term risk of long space missions (Table 4).[51,52] In parallel to the spatial activity of USA and USSR, radiobiology data from ground accelerators pointed out during the same period that the relative biological effectiveness of high-energy particles is much higher than X- and gamma-rays and that the DNA and chromosome damage induced by heavy ions are much more severe. These conclusions will be taken seriously into consideration during the MIR and ISS missions.[53]

The Mir space station. Progressively built from different modules from 1986 to 1996, Mir was disintegrated into atmosphere in 2001. Like the other space stations, Mir completed about 16 orbits per day. Crew dosimetry was mainly carried out using thermoluminescent dosimeters. The dose received during the different missions is summarized in Table 3. The dose rates were similar to the previous space missions described above, despite higher values for Mir-13, Mir-14 and Mir-15, probably due to solar flares (Fig. 5).

Testard *et al*. (1996) and Obe *et al*. (1997) applied Giemsa techniques to blood samples from cosmonauts during Mir missions: while there was no significant link between the flight duration and cytogenetic damage for missions of shorter than 30 days, a tendency to an increase of chromosome breaks and aberrations was observed after flights of more than 180 days.[54,55] For these long flights, a great heterogeneity between individuals was noticed. Furthermore, a higher frequency of multi-aberrant (or rogue) cells showing several chromosome breaks was observed at an unexpected rate.[54,55] However, again, the impact of these results was limited by the low number of mitoses per cosmonauts and by the precision of the technique itself.

In the 1980's, the fluorescence in situ hybridization (FISH) technique progressively replaced the Giemsa technique. The considerable advantage of FISH technique is the possibility to use fluorescence probes that hybridize specific or whole part of given chromosomes, enabling a more precise determination of translocations, exchanges and later, centromeres and telomeres. However, the introduction of this new technique did not alleviate the problem of statistical significance, specifically for low-dose data.[56] Furthermore, the number of cosmonauts and astronauts performing successive missions increased, notably those who participated to Salyut and Mir and thereafter to Mir and ISS missions. This new deal added therefore extra-complexity in the conclusions drawn from cytogenetic data by introducing biases due to experienced and non-experienced crews, cumulative doses and impact of inter-flights periods. Furthermore, unlike the Apollo observations, some experienced crews showed less chromosome damage than expected if a simple linear relationship between accumulation of damage and cumulative time in space was applied. Such results raised therefore the interesting radiobiological problem of radioadaptation: how to account for a simple and universal dose-response with such variety of situations? (Table 4).

Starting from the mid-1990s, a new space experiment, SilEye, was designed to clarify the eye flashes issues. Two active particles detectors based on silicon technology were installed on MIR. SilEye-1 was launched in 1995 to investigate the hypothesis that heavy ions are the dominant source triggering eye flashes.[57] To improve quality and quantity of measurements, SilEye-2 was placed in MIR in 1997. SilEye2 detected a total of about 116×10^3 protons and 858 nuclei with $Z \geq 2$ in the 814 min of observation. For the same time, 116 eye flashes were perceived. The authors found a significant increase of probability that particles give rise to eye flashes for LET above 10 keV.μm^{-1}.[58] The rate of eye flashes in the SAA was about twice that of assessed

outside the SAA, strongly suggesting the impact of secondary particles in the eye flashes occurrence. Analysis of data collected with the Sileye-2 experiment suggested that there are at least two different components of cosmic rays that cause eye flashes: heavy nuclei, via a direct ionization, and protons, via an indirect process. It is noteworthy that the average eye flashes rate during skylab in 1974 was about 10 times higher than during the MIR and Apollo-Soyuz tests. These differences are still unclear, but these three spacecrafts show different material composition, raising again the question of nuclear interaction between metallic stuff and GCR particles.[59)]

The International Space Station (ISS). Coordinated by NASA, the International Space Station (ISS) is a collaborative research project with European, Japanese, and Canadian space agencies. The ISS was launched in 1998 and might reach its final form in 2011 with about 15 modules including American Freedom, the Russian Mir-2, the European Columbus and the Japanese Kibo. The ISS is the biggest man made structure ever put into low Earth orbit. Similarly to the other space stations, the ISS completes about 15.7 orbits per day.

In addition to the developments of the FISH technique (M-FISH, Q-FISH, etc), radiobiologists became aware that the time required for getting metaphases may be a source of biases. Hence, techniques allowing the premature condensation of chromosome (PCC) were applied to lymphocytes of astronauts together with Giemsa and FISH.[56,60,61)] Despite significant differences in protocols and discrepancy between research groups, cytogenetic data from Mir, Space Shuttle and ISS strongly suggested that the frequency of total chromosome aberrations are higher post-flight than pre-flight, notably after flights longer than 180 days. The occurence of rogue cells becomes more and more frequent as far as the flight duration increases (Table 4). However, additional conclusions were drawn about the fate of the chromosome aberrations long times after flight and between two flights: 1) the yield of chromosome aberrations decreases some years after a first flight but without reaching the unirradiated values; 2) a second flight does not increase in proportion the yield of aberrations, suggesting a non-additive or even and infra-additive effect. These conclusions raise again the possibility of a radio-adaptive response (Table 4).

In addition to developments of FISH, the determination of DNA damage, their repair and their signaling became more and more precise with the use of immunofluorescence technique with antibodies against the phosphorylated forms of the histone variant H2AX (γ-H2AX). The γ-H2AX immunofluorescence technique revolutionized our estimation of the radiation-lethal events since it allows the determination of each individual DNA double-strand break (DSB) inside cell nuclei with a one-to-one correlation between DSB and γ-H2AX foci.[62)] Recently, Ohnishi *et al*. presented a very exciting work consisting in maintained human cells frozen during all along a 133 days ISS mission. Interestingly, cells thawed and cultured after the flight revealed about 1.5 tracks of γ-H2AX foci per 100 nuclei. The total absorbed dose during the flight was 43.5 ± 2.8 mGy corresponding to a dose rate of 327 μGy per mission day, in very good agreement with the values described above.[63)] The presence or the absence of highly damaged (rogue) cells was however not mentioned. Since low-LET radiations (X, electrons, protons) produce more homogenously dispersed γ-H2AX foci in nuclei, the authors suggested logically that tracks may have been caused by high-LET ions.[63)] However, the relative contribution to the total dose of particles with LET more than 10 keV per μm was 6.2%. The experiments of the Ohnishi's group belong to a very ambitious research program investigating notably the impact of p53 status on the space radiation exposure.[64,65)]

As described above, the eye flashes occurrence was still not fully understood at the end of the 1990's.[66–69)] A continuation of the SilEye studies, under the name ALTEA (Anomalous Long Term Effects on Astronauts), was planned on ISS. The Alteino/SilEye3 was the first experimental achievement of the program providing the first measurement with full Z discrimination of the radiation environment in the ISS.[67,70)] Part of ALTEA experiment consisted of a helmet for astronauts containing six particle detectors that can map the cosmic rays that pass through the astronaut's. The measured rate of ions in the eye produced an average rate of 5×10^{-2} eye flashes per min (20 in about 420 min of observation). Preliminary results seem to indicate that the most probable ions for generating eye flashes are light ions.[70)]

ADVANCES IN RADIOBIOLOGY CONCERNING SPACE RADIATION

Space radiation biology: the general point of view of the radiobiologist

Comparison with medical exposure. A dose of about 400 μGy per space mission day is 5000 times lower than a radiotherapy fraction (2 Gy), 10–20 times lower than a mammography session (2 × 2 mGy) or than an abdominal CT scan (8 mGy). We have seen above that the average dose rate during missions is about 0.28 $\mu Gy.min^{-1}$. Such dose rate is about 10^8 times lower than medical exposure but in the same order than natural radioactivity. Space radiation exposures raise some radiobiological questions in common with radiodiagnosis:

- 1) is there any risk of mutagenesis and radiation-induced cancer after low-dose?
- 2) what is the nature of the low-dose-induced deterministic events?
- 3) is there any radiobiological effect related to repeated doses?
- 4) what is the impact of the adaptive response in the final outcome?

The radiation-induced cancer risks. Since the 1990's, an increasing body of data has suggested a non-linear threshold (NLT model) that postulates that low-dose radiation is harmless below a threshold of about 100–200 mGy, which contradicts the linear non-threshold (LNT) model supported by the International Commission for radiation protection (ICRP).[71] On the arbitrary basis of 400 μGy per mission day and without considering any adaptive response, these threshold values are only expected for continuous or cumulated 250–500 mission days, at least. Among all the 500 space crewmen, only 30 (6%) and 8 (1.6%) performed space missions of more than 250 and 500, respectively. Less than ten (2%) cosmonauts participated to continuous missions longer than 250 days. Mir still holds the record of the longest manned periods in space: Valery Polyakov stayed 437.7 days onboard and Sergei Krikalev has spent 2.2 years in space throughout six flights. The theoretical cumulated total dose received by these two cosmonauts is 175 and 321 mGy, respectively (Fig. 6). These data suggest that the cohort of spacecraft crews is still not large enough and long-term missions are still not frequent enough to estimate rigorously an eventual risk of space radiation-induced cancer. Hence, it is therefore important to propose the development of an open general register of all the international spacecraft crews with detailed radiation exposure histories, in order to better define relevant biological endpoints and irradiation protocols close to spatial reality.

The radiation-induced cataracts. Among the non-cancer events induced by radiation, cataracts appear to be a possible consequence of the exposure to space radiation. Forty-eight (16.2%) cases of lens opacification were observed in the 295 astronauts of the NASA's Longitudinal Study of Astronaut Health (LSAH) project. An increased risk of cataract was generally observed after doses higher than 8 mSv.[72,73] Interestingly, the radiation-induced cataract has long been considered as a quite rare pathology, requiring a threshold dose roughly estimated at 2 Gy to the lens. Recent data suggest that the threshold dose might be much lower than 2 Gy,[74,75] suggesting also that the definition of deterministic and stochastic events as defined by ICRP publications are now questionable.[76,77] These data make therefore possible the occurrence of space radiation-induced cataracts for missions as short as 20 days. Like for cancer risks, further investigations about radiation-induced deterministic events are needed to provide a better definition of non-linear biological effects at low doses. Besides, it is noteworthy that the link between eye flashes phenomenon and cataracts is still misknown and needs also to be clarified.

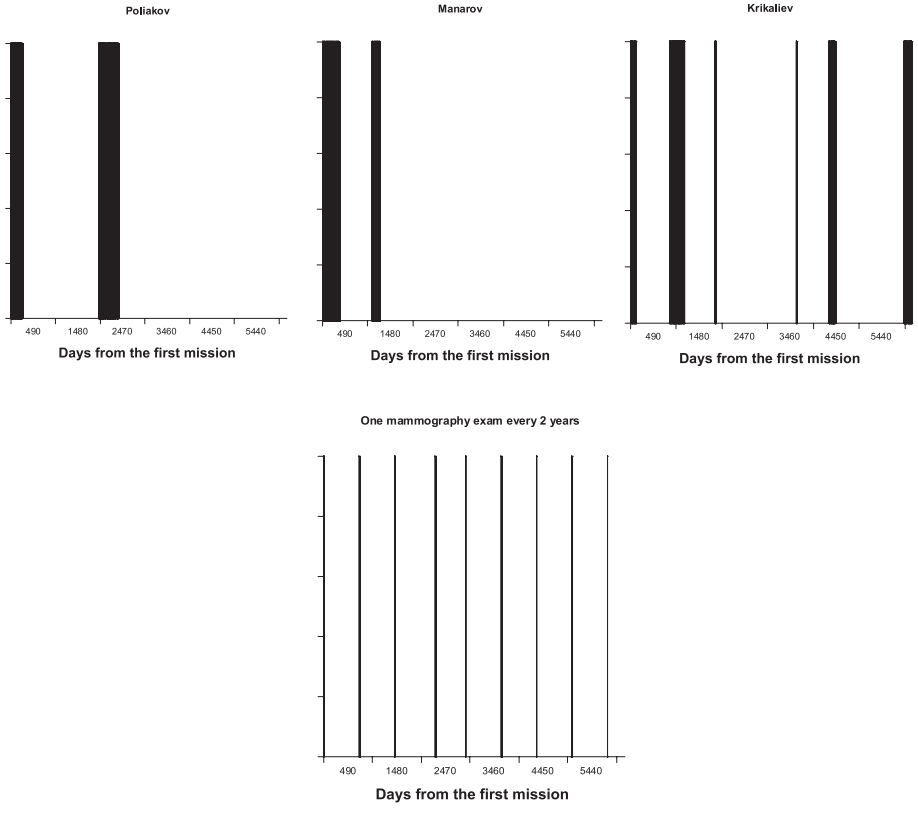

Fig. 6. Schematic illustration of radiation exposure history of some cosmonauts and patients subjected to one mammography irradiation every two years.

Space radiation biology: the specific cellular and molecular effects

Non-linear effects at low-dose. The two above sections concerned macroscopic events (cancer and cataracts). Let's examine now the radiobiological phenomena that are specific to an exposure to low-dose and of spatial interest. The number of radiation-induced DNA damage, directly linked to physical energy deposition, is systematically proportional to the dose. Conversely, clonogenic survival obeys a linear-quadratic function of the dose.[78,79] The phenomenon of hypersensitivity to low dose (HRS) followed by induced radioresistance (IRR) is also a representative example of a non-linearly dose-dependent event (Fig. 7). In fact, HRS is generally observed between 1 mGy and 50 cGy with a maximum peak at a cell-line-dependent threshold of about 10–30 cGy.[80–82] At this peak, clonogenic survival may reach similar values as for doses of 1–3 Gy (*i.e.* at doses about 100 times higher!). Similarly, an inverse dose rate effect was shown in cells showing marked HRS responses at dose rate lower than $mGy.min^{-1}$.[83,84] Lastly, cells at the vicinity of irradiated cells may show bystander effect that may correspond to an HRS-compatible dose. Hence, although the link between HRS and bystander requires further investigations, the HRS phenomenon, in all its forms, may concern space radiation exposure. Although a number of molecular models have been proposed, mechanisms of HRS are however unclear.[80–82] It has been suggested that HRS may depend upon changes in chromatin conformation,[80] failure of the ATM-dependent G_2/M checkpoint,[81] or DNA repair defects.[85] More recently, we have shown that HRS occurrence requires three conditions, at least : 1) an impairment of the predominant DNA double-strand breaks (DSB) repair pathway specific to low-dose, 2) an impairment of the rescue (recombination) DSB repair pathway under particular genetic conditions; 3) an impairment of the radiation-induced of cell cycle arrest due to particular genetic conditions.[86] Interestingly, during our investigations, cells with an unexpectedly high number of DSB have been observed between 1 mGy to 30 cGy. These so called "highly damaged cells" (HDC) might correspond to the multi-aberrant (rogue) cells observed with cytogenetics. The HRS phenomenon may also explain the excess of radiation-induced cataracts at low-dose since similar cellular effects are observed between 1 mGy to 30 cGy and at doses 100 times higher. However, it must be stressed that HRS occurs more likely in tumorigenic cells and rarely in quiescent skin fibroblasts.[86,87]

Calculations about the space radiation-induced DNA damage. Since nearly 20 years, there is increasing evidence that non-repaired radiation-induced DSB are correlated to cellular death and mis-repaired radiation-induced DSBs are correlated to genomic instability.[78,79] Ionizing radiation produce a large spectrum of energy microdepositions, that, through excitations and ionisations, lead to the formation of free radicals species that generate three major types of DNA damage (base damage (BD), single-strand breaks (SSB) and DSB.[78,79,88] The formation of DSB requires more than about 100 eV deposed per nm^3 whereas BD require about 100 times less energy density.[89] One Gy X-rays produces 10000 BD, 1000 SSB and 40 DSB per human diploid cell. The more energy required for the DNA damage formation, the longer time required for its repair. In human diploid cells, 50% of DSB are repaired in about 1 h whereas most of BD generally show a repair half-time of less than ten minutes.[78,79,88,90] In addition, during their repair, BD are generally converted into SSB in some minutes post-irradiation. Hence, it is frequent to observe an over-production of additional SSB after tens min post-irradiation, which affects chromatin condensation.[90] If one considers that secondary

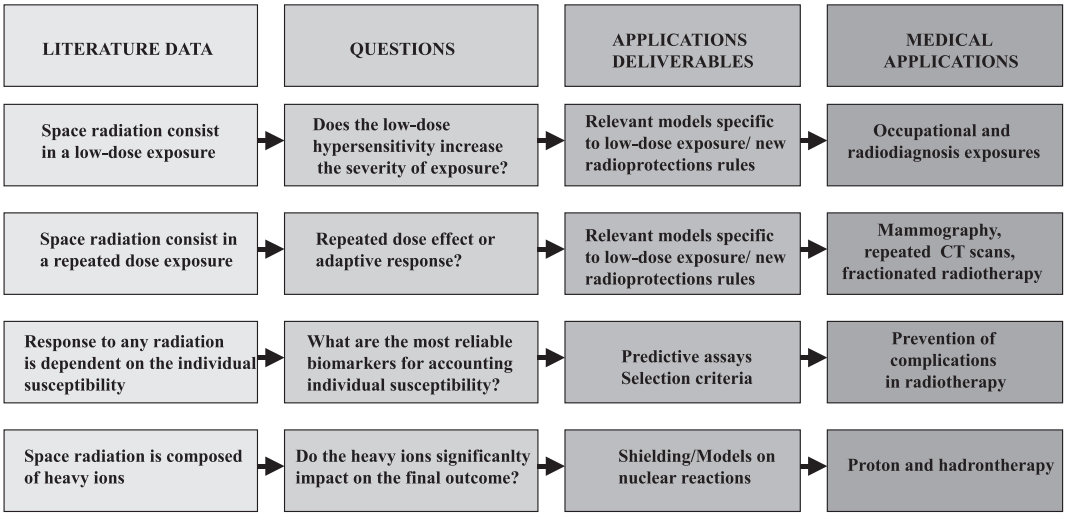

Fig. 7. Summary of the questions about new aspects of space radiation biology raised in this review.

protons and electrons roughly produce the same range of DNA damage as photons, 400 μGy per mission day would correspond to about 4 BD per cell, 4 SSB every 100 cells and 16 DSB every 1000 cells. These values correspond to 3 BD per 10 cell, 3 SSB per 100 cells and 1 DSB every 1000 cells every passage in SAA (about 15 passages for 15 min per day). These values are negligible by comparison with DNA damage due to spontaneous stress. However, one can imagine two different scenarios: 1) the SSB are not repaired before the second passage in the SAA: chromatin decondensation increases the severity of the damage induced thereafter. As a result, severe DNA damage accumulate at each passage in the SAA; 2) the first passage in the SAA triggers radio-adaptation process and reduces the biological effect of the next one(s). As a result, the number of DNA damage decrease at each passage in the SAA. Let discuss the relevance of these two scenarios (Fig. 7).

The repeated doses and the chromatin decondensation. Preliminary results from our lab indicate that X-rays DSB induced after 1 Gy + some minutes + 1 Gy are repaired more slowly than after 2 Gy in human primary fibroblasts. Similar observations have been performed on doses up to 2 mGy (Viau *et al*., in preparation). Between the two doses, during some minutes, there is no significant repair of DSB, but initial SSB may be repaired and secondary SSB due to BD excision can appear and increase the chromatin decondensation. Such a chromatin decondensation may make slower the DSB repair process and leads to the accumulation of unrepaired DSB.[91] Hence, it is possible that few DSB surrounding by SSB may have non negligible biological consequences. Such situation may occur in the particular case of space radiation exposure and leads to chromosome breaks and eventually to the production of multi-aberrant (rogue) cells. Some other manifestations of the deleterious effect of repeated doses have been published. The so-called "W" effect is one of the most representative examples.[92,93] From a linear electron accelerator providing pulsed irradiation separated by time intervals of a second to few minutes, the V. Favaudon's group observed a tetraphasic, W-shaped time-dependent dose-response curve strongly dependent on the cell line.[92,93] Although this W effect was not explored in the mGy range, its existence provides new insights in the knowledge of multiphasic phenomena induced by repeated doses.

The adaptive response phenomenon. The previous scenario considered that the repair rate of DSB is not dependent on the previous doses and that chromatin decondensation makes slower the DSB repair rate. Conversely, one can imagine that experienced cells may repair more efficiently after a priming (or *conditioning*) dose. The adaptive response phenomenon is defined as an increased radioresistance observed at a *challenging* exposure to high-dose of more than 1 Gy due to a conditioning exposure to low-dose (0.001–200 mGy) generally delivered at dose rate higher than 100 $\mu Gy.min^{-1}$ and with an interval period (4–6 h) between the priming dose and the challenging dose.[94,95] A number of examples of adaptive response exist in literature. However, care must be taken with intercomparisons since a great variety of cellular models and irradiations protocols have been applied.[96–99] Since the challenging dose (more than 1 Gy) has not been encountered for human exposure in space, the adaptive response phenomenon would concern only space crewmen who would be eventually exposed to a medical irradiation after a flight, which is not probable. Furthermore, the dose rate of the priming dose, the periodicity and the duration of the passage above the SAA are very different from the conditions required for the occurrence of the adaptive response. Hence, the literature data suggests *to date* that space radiation exposure cannot be concerned by the adaptive response phenomenon as it was defined above. However, how to understand that experienced crews may show less chromosome aberrations than non-experienced crews? First, it must be reminded again that the interval of time required for seeing the decrease of aberrations has nothing in common with the adaptive response (1–2 years between two flights versus 4–6 h between two doses). Second, it must be stressed that inter-individual differences appeared nearly always higher than differences between the pre- and the post-flight data (Table 4). Since space radiation exposure fits well to the definition of priming dose range, some authors have however investigated the fate of cells exposed in space during a flight and irradiated thereafter at high dose on Earth. This is notably the case of Takahashi *et al*. who have exposed two human lymphoblastoid cell lines for 133 days in ISS and, once returned to Earth, cultured for 6 h and exposed them to a challenging X-ray dose of 2 Gy. The authors concluded on an adaptive response that would be dependent on the p53 status.[100] Hence, despite interesting studies using space radiation as a tool for investigating the basic mechanisms of adaptive response phenomenon, further investigations are needed to evaluate the potential impact of this phenomenon in space radiobiology.

Impact of individual susceptibility to radiation. The fact that each individual may provide a specific response to radiation has been observed very early, notably some years after the discovery of X-rays by Roentgen.[101,102] However, the notion of intrinsic radiosensitivity appeared in the 1980's with the accumulation of the clonogenic survival data. In 1981, a correlation between clinical responsiveness (quantified *in vivo* by tumour local control) and intrinsic radiosensitivity (assessed *in vitro* by clonogenic assays) was established[103] and a first classification was proposed in 1996.[104] Unfortunately, although many works on genetically modified yeasts and rodents have undoubtedly contributed to increase our knowledge in DNA damage repair and signalling and in the control of genomic stability, they erased the notions of the continuum of responses to radiation in the minds of many radio- therapists and -biologists. Worse, the radiosensitivity with non-human models was generally pro-

vided from mutations of proteins whose *identical* homologues did not exist in humans (e.g. Ku70, Ku80, Rad51, Rad52, etc). Finally, the obvious necessity to quantify and describe each level of severity of radiation responses disappeared behind a number of "monogenic" studies that represent, still to date, the great majority of papers in radiobiology. A number of genetic syndromes have been associated with radiosensitivity. This is notably the case of ataxia-telangiectasia (*ATM* mutations), Nijmegen Breakage syndrome (*NBS1* mutations), ataxia telangiectasia like-disorder (ATLD) (*MRE11* mutations), Fanconi anemia (*FANC* genes mutations), Bloom's syndrome (*BLM* mutations), Xeroderma Pigmentosum (*XP* genes mutations), Cockayne syndrome (*CS* genes mutations), and mutations of ligase I (*LIG1*) and ligase IV (*LIG4*).[105] Obviously, because of the intensity of their specific acute tissue reactions and their very low frequency, the great majority of these syndromes are detected at the early ages and do not concern the spacecraft crews. However, some heterozygous mutations of the genes described above are frequent and may confer a very moderate radiosensitivity and eventually cancer-proneness. As an example, while they are not systematically associated with obvious clinical signs, the heterozygous ATM mutations may represent 1% of world population and have been linked to higher risk of cancer.[106] These mutations are also representative examples of some actual confusion in literature: they are linked to both individual susceptibility to radiosensitivity *and* cancer risk. However, the molecular and cellular endpoints correlated to these two different notions may be very different. The next section will present briefly the important of the choice of the biomarkers in order to predict quantitatively either radiosensitivity (radiotoxicity) or genomic instability (cancer proneness).

The question of high-LET particles. The relative abundance of GCR is inversely proportional to the nuclear charge Z. For example, while protons and He ions represent 85% and 14% of the baryonic GCR, respectively, the Fe and Ni ions contributions are 10^4 and 10^6 times smaller.[16,25] The linear energy transfer (LET) of heavy charged particles may range from 100 to 2000 keV.μm^{-1}. However, the relative biological efficiency (RBE) of such particles never exceeds a factor of 10 for LET of about keV.μm^{-1}.[107] Furthermore, it is noteworthy that for larger LET, the overkill phenomenon decreases RBE. Consequently, the potential toxicity of heavy ions of high LET never compensate their low relative abundance, even by applying radiation quality weighting factors. However, by considering impacts equally probable, the contribution of heavy ions to the dose may be substantial but remains far from the relative contribution of protons.[108] These conclusions are not fully consistent with the frequency of eye flashes and the impacts of Ni ions on helmets of Apollo astronauts that have been attributed to heavy ions. These data raise therefore the question of a possible underestimation of heavy ions that might be produced through nuclear interactions with high-energy protons with spacecrafts materials, and notably metallic stuff (Fig. 7). Further investigations are required to better evaluate the real effect of heavy ions in the final response to space radiation exposure. This question is inasmuch important as heavy ions may produce non-targeted and delayed biological effects that would contribute significantly to the final outcome, although a consensual mechanistic model to explain bystander phenomena is still needed.[109]

Space radiation biology: practical considerations

Biomarkers to account for individual susceptibility and space radiation response. What is the most relevant endpoint(s) to predict response to space radiation: gene expression, gene mutations or gene functions? To date, genomics technologies have provided a plethoric amount of data and emphasized the potential impact of gene mutations and expression in radiation response while the quantitative correlation between radiosensitivity and these endpoints still remains to be established. Hence, while single nucleotide polymorphisms (SNP) may account for certain cases of cancer proneness, there is still no quantitative general correlation between SNP and radiosensitivity. Moreover, some patients showing the same SNP may exhibit different degrees of severity of tissue reaction.[110–112] Similarly, further investigations are required to generalize the predicting power of gene expression for many radiobiological features: the major proteins involved in the radiation response are so abundant that there is no change in expression in a large range of radiation dose.[23] In parallel, the study of radiobiological features of some human genetic syndromes has consolidated the conclusion that predictive assays based on the *functionality* of the DNA repair pathways involved in the response of radio- and chemo-therapy may permit to quantify and prevent the over-acute reactions to treatment.[79] Recently, from a collection of human cells representing the largest spectrum of radiosensitivity investigated and by testing the most extensively used molecular assays susceptible to predict radiosensitivity, we pointed out: 1) a quantitative correlation between survival fraction at 2 Gy (SF2) and the yield of unrepaired DSB, the major radiation-induced DNA damage, generally managed by the DNA-PK dependent pathway; 2) the existence of a new DSB repair pathway (dependent upon the MRE11 protein) active in quiescent tissues and whose lack of control may explain genomic instability (i.e. cancer proneness) but also some moderate radiosensitivities.[79] From these findings we proposed therefore to apply a double (DNA-PK and MRE11), at least, predictive assay based on the determination of the DSB repair functionality to *predict any level of radiosensitivity.* Lastly, a classification have been proposed in which group I gathers individuals showing radioresistance, group II gathers individuals with moderate radiosensitivity but high cancer proneness and group III gathers individuals showing very

high radiosensitivity.[79] After a systematical analysis of more than 200 cases, it appears that group I represents about 75% of cases and group II about 15–20% of cases (Granzotto *et al*, in preparation). Hence, such predictive assays may initiate a selection system of space crewmen on the basis of their individual susceptibility to radiation (Fig. 7).

Blood in question? At this stage, it must be stressed that all the cytogenetics studies concerning space radiation biology have been performed with lymphocytes (Table 4). However, Testard and Sabatier have highlighted that lymphocytes is not the ideal cellular model and some technical artifacts may bias data interpretation.[56] The great majority of investigations in both gene expression and gene mutations are performed with blood cells, as well. However, post-irradiation tissue reactions do not concern blood cells only and there is no correlation between radiobiological data provided by blood cells and those provided by conjunctive tissues from the same individual. Indeed, in 1990, Kushiro *et al*. did not found any correlation between radiosensitivity of fibroblasts and lymphocytes.[113] In 1991, from quiescent skin and blood cells of 34 non-radiosensitive donors, Green *et al*. demonstrated significant differences in radiosensitivity for fibroblasts but not for T-lymphocytes. There was no correlation in radiosensitivity between the two cell types.[114] In 1992, from 30 human fibroblasts and 29 lymphocytes, Geara *et al*. concluded that there was no correlation between survival fractions assessed in lymphocytes and those assessed in fibroblasts.[115] In addition to these data, it must be stressed that blood cells are naturally more prone to apoptosis than fibroblasts, that the size of nuclei of lymphocytes are about 3–4 times lower than that of the other cell types of the conjunctive tissue, probably due to a more condensed chromatin. Lastly, since circulating lymphocytes cannot be preserved and amplified for long periods, they do not present the advantage of tissue like skin that can be established as primary cells and kept in liquid nitrogen.[23] Hence, a certain care must be taken with radiobiological data from blood cells. Other tissue models like fibroblasts may be more relevant to predict some radiobiological features.

CONCLUSIONS

In this review, we have deliberately focused on space radiation effects on human cells. This mainly concerns cytogenetic data, eye flashes and cataracts occurrence (Fig. 1). The results accumulated from micro-organisms and rodent models, as well as the potential effect of weightlessness has been omitted to avoid any over-interpretations and extrapolations. The picture of the space radiation effects on human cells in 2011 may be summarized in 3 points:

- the quantification of the dose received by spacecraft crews and the energy spectrum of space particles become more and more precise. However, further investigations about secondary particles, although made difficult by the complexity of spacecraft geometry and the diversity of materials used, are still required, especially for ions.
- the diversity of radiation history of each astronauts and their individual susceptibility make very difficult any epidemiological analysis for estimating hazards due to space radiation exposure. Maybe a more intense international collaboration may help in overcoming these difficulties.
- cytogenetic data undoubtedly revealed that space radiation exposure produces significant damage in cells. However, our knowledge of the basic mechanisms specific to low-dose, to repeated doses and adaptive response is still poor. Furthermore, experiments about genomic instability and delayed mutagenesis may help in quantifying the risk of potential space radiation-induced cancer. The application of new radiobiological techniques may help in progressing in this field.

REFERENCES

1. White RJ and Averner M (2001) Humans in space. Nature **409**: 1115–1118.
2. Cucinotta FA and Durante M (2006) Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings. Lancet Oncol **7**: 431–435.
3. Durante M and Cucinotta FA (2008) Heavy ion carcinogenesis and human space exploration. Nature Review Cancer **8**: 465–472.
4. Durante M (2002) Biological effects of cosmic radiation in low-Earth orbit. Int J Modern Phys A **A17**: 1713–1721.
5. NCRP (2006) Information needed to make radiation protection recommendations for space missions beyond low-earth orbit. Report N.153. National Commission for Radiation Protection; Bethesda, MD, USA.
6. Parker EN (2006) Shielding space travelers. Scientific American **294**: 40–47.
7. NASA (2009) Human health and performance risks for space exploration missions. NASA-SP-2009-3405.; Houston, TX, USA.
8. ESA (2003) HUMEX: Study in survivability and adaptation of humans to long-duration interplanetary and planetary environments. ESA-ESTEC/SP-1264. European Space Agency.
9. NAS (2006) Space Studies Board. Space radiation hazards and the vision for space explorations. National Academy of Sciences of USA. National Academy Press.
10. Held KD (2009) Effects of low fluences of radiations found in space on cellular systems. Int J Radiat Biol **85**: 379–390.
11. Lear J (1965) Kepler's dream. Univ. of California Press; Berkeley, USA.
12. Todd P, *et al* (1974) Current topics in space radiation biology. In: Tobias C, Todd P eds. Space radiation biology and related topics. pp. 1–18. Academic Press; New York and London.
13. Millikan RA (1947) Electrons (+ and –), protons, photons, neutrons, mesotrons and cosmic rays. University of Chicago Press; Chicago, Illinois, USA.
14. Hess VF and Eugster JAG (1949) Cosmic radiation and its

biological effects. Fordham Univ. Press; New York.
15. Johnson TH (1939) Evidence that protons are the primary particles of the hard component. Review Modelling Physics **11**: 208–210.
16. Haffner JW (1967) Radiation and shielding in space. In. pp. Academic Press; City.
17. Freier P, *et al* (1948) Evidence of heavy nuclei in the primary cosmic radiation. Physics Reviews **74**: 213–217.
18. Van Allen JA and Franck LA (1959) Radiation around the earth to a radial distance of 107,400 km. Nature **183**: 430.
19. Van Allen JA and Franck LA (1959) Radiation measurements to 658,300 km with Pioneer IV. Nature **184**: 219.
20. Singer SF (1958) Trapped albedo theory of the radiation belt. Physics Reviw Letter **1**: 181.
21. Neugebauer M and Snyder CW (1964) Interplanetary solar wind measurements by Mariner II. In: Muller P eds. Space Research. pp. 89–113. North-Holland Publications; Amsterdam.
22. Winckler JR, *et al* (1961) The time variation of solar cosmic rays during July 1959 at Minneapolis. J Geophys Res **66**: 995–1022.
23. Joubert A and Foray N (2006) Repair of radiation-induced DNA double-strand breaks in human cells: history, progress and controversies. In: Landseer Br eds. New Research on DNA Repair. pp. Chapter 10. Nova Science Publishers, Inc.; Hauppauge N. Y.
24. Comstock GM, *et al* (1969) Energy spectra and abundances of the cosmic-ray nuclei helium to iron from the OGO-1 satellite experiment. Astrophysics Journal **155**: 609–617.
25. Benton ER and Benton EV (2001) Space radiation dosimetry in low-Earth orbit and beyond. Nucl Instrum Methods Phys Res B **184**: 255–294.
26. Bethe HA and Ashkin J (1953) Passage of radiations through matter. In: Segre E eds. Experimental Nuclear Physics. pp. Wiley; New York.
27. Bertini HW (1963) Low-energy intranuclear cascade calculation. Physics Review Letter **131**: 1801–1821.
28. Jorgensen AM, *et al* (2007) Passive radiation shielding considerations for the proposed space elevator. Acta Astronautica **60**: 198–209.
29. Durante M (2008) Physical and biomedical countermeasures for space radiation risk. Z Med Phys **18**: 244–252.
30. Pugliese M, *et al* (2010) Tests of shielding effectiveness of Kevlar and Nextel onboard the International Space Station and the Foton-M3 capsule. Radiat Environ Biophys **49**: 359–363.
31. Spillantini P, *et al* (2007) Shielding from cosmic radiation for interplanetary missions: active and passive methods. Radiation Measurements **42**: 14–23.
32. Curtis SB (1974) Physics and dosimetry. In: Tobias Ca, Todd P eds. Space radiation biology and related topics. pp. 21–99. Academic Press; New York.
33. Schneider M and Janni JF (1969) A comprehensive summary of dose rate measurements abpard the fourth and sixth Gemini flights in The current experimental approach to the radiological problem of space flight (J.F. Janni and F.E. Holly, eds). Aerosp Med **40**: 1535–1546.
34. Bailey JV, *et al* (1977) Radiological protection and medical dosimetry fro the Skylab. Chapter 9. In: Dietlein Rsjalf eds. Biomedical results from Skylab NASA SP 377. pp. NASA; Washington.
35. Fenech M (2000) The in vitro micronucleus technique. Mutat Res **455**: 81–95.
36. Grote SJ, *et al* (1981) Observations of radiation-induced chromosome fragment loss in live mammalian cells in culture, and its effect on colony-forming ability. Int J Radiat Biol Relat Stud Phys Chem Med **39**: 395–408.
37. Bender MA, *et al* (1968) The Gemini XI S-4 spaceflight-radiation interaction experiment: the human blood experiment. Radiat Res **34**: 228–238.
38. Bender MA, *et al* (1967) The Gemini-3 S-4 spaceflight-radiation interaction experiment. Radiat Res **31**: 91–111.
39. Kovalev EE and Grigoriev YG (1970) Effects on the organism of radiation energy of cosmic space. In: Ussr Aoso eds. Bases of cosmic biology and medicine. pp.
40. Parin VV, *et al* (1969) Characteristics of biological effects of cosmic radiation, model investigations. In: Favorite Wvafg eds. Life Sciences and Space Research. pp. 160–170. North Holland; Amsterdam.
41. Kimzey SL, *et al* (1975) Hematology and Immunology Studies. In: Johnston Rs, Dietlein Lf, Berry Ca eds. Biomedical results of Apollo NASA SP368 Report Section III Volume III. pp. Bio Technology, Inc.; New York.
42. Johnston RS and Dietlein LF (1977) Biomedical results from Skylab. NASA SP377. NASA; Washington.
43. Budinger TF, *et al* (1977) Light flash observations. Experiment MA-106. Volume 1. Astronomy, Earth Atmosphere and Gravity Field, Life Sciences, and Materials Processing. In: Center Lbjs eds. Apollo-Soyuz Test Project Summary Science Report, NASA Special Publication NASA SP-412. pp. 193–209. NASA; Washington.
44. Lockhart LH (1977) Cytogenetic studies of blood (Experiment M111).Chapter 24. In: Johnston Rs, Dietlein Lf eds. Biomedical results from Skylab NASA SP 377. pp. NASA; Washington.
45. Kozlova SB, *et al* (1978) [Measurement of the radiation doses in the area of the inner radiation belt of the Earth]. Kosm Biol Aviakosm Med **12**: 85–87.
46. Markelov VV and Chernykh IV (1982) [Radiation control on Saliut stations using directly indicating dosimeters]. Kosm Biol Aviakosm Med **16**: 81–84.
47. Red'ko VI, *et al* (1974) [Dosimetric studies on the orbital cosmic station "Saliut"]. Kosm Biol Aviakosm Med **8**: 3–6.
48. Gagarin Yu F, *et al* (1998) Heavy particle fluxes in Salyut space station orbit. Adv Space Res **21**: 1699–1702.
49. Bogomolov AV, *et al* (1998) Energetic neutron and gamma-ray spectra under the earth radiation belts according to "SALYUT-7" [correction of "SALUTE-7"]-"KOSMOS-1686" orbital complex and "CORONAS-I" satellite data. Adv Space Res **21**: 1801–1804.
50. O'Neill JP, *et al* (1987) Refinement of a T-lymphocyte cloning assay to quantify the in vivo thioguanine-resistant mutant frequency in humans. Mutagenesis **2**: 87–94.
51. Khaidakov M, *et al* (1999) Study on genotoxic effects of the space environment: a comparison between experienced cosmonauts and unexposed Russian twins. Mutat Res **430**: 337–

342.
52. Khaidakov M, *et al* (1997) Molecular analysis of mutations in T-lymphocytes from experienced Soviet cosmonauts. Environ Mol Mutagen **30**: 21–30.
53. NCRP (1989) Guidance on radiation received in space activities. National Commission for Radiation Protection; Bethesda, MD, USA.
54. Testard I, *et al* (1996) Radiation-induced chromosome damage in astronauts' lymphocytes. Int J Radiat Biol **70**: 403–411.
55. Obe G, *et al* (1997) Chromosomal aberrations in blood lymphocytes of astronauts after long-term space flights. Int J Radiat Biol **72**: 727–734.
56. Testard I and Sabatier L (1999) Biological dosimetry for astronauts: a real challenge. Mutat Res **430**: 315–326.
57. Bidoli V, *et al* (2000) Study of cosmic rays and light flashes on board Space Station MIR: the SilEye experiment. Adv Space Res **25**: 2075–2079.
58. Avdeev S, *et al* (2002) Eye light flashes on the Mir space station. Acta Astronaut **50**: 511–525.
59. Casolino M, *et al* (2003) Study of the radiation environment on MIR space station with SILEYE-2 experiment. Adv Space Res **31**: 135–140.
60. Ritter S and Durante M (2010) Heavy-ion induced chromosomal aberrations: A review. Mutat Res **174**: 14–19.
61. Obe G and Durante M (2010) DNA double strand breaks and chromosomal aberrations. Cytogenet Genome Res **128**: 8–16.
62. Rothkamm K and Lobrich M (2003) Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci U S A **100**: 5057–5062.
63. Ohnishi T, *et al* (2009) Detection of space radiation-induced double strand breaks as a track in cell nucleus. Biochem Biophys Res Commun **390**: 485–488.
64. Takahashi A, *et al* (2010) The first life science experiments in ISS: reports of "Rad Gene"- Space radiation effects on human cultured cells. Biol Sci Space **24**: 17–41.
65. Takahashi A, *et al* (2010) The expression of p53-regulated genes in human cultured lymphoblastoid TSCE5 and WTK1 cell lines during spaceflight. Int J Radiat Biol **86**: 669–681.
66. Fuglesang C, *et al* (2006) Phosphenes in low earth orbit: survey responses from 59 astronauts. Aviat Space Environ Med **77**: 449–452.
67. Casolino M, *et al* (2003) Space travel: Dual origins of light flashes seen in space. Nature **422**: 680.
68. Sannita WG, *et al* (2007) Electrophysiological responses of the mouse retina to 12C ions. Neurosci Lett **416**: 231–235.
69. Sannita WG, *et al* (2004) Effects of heavy ions on visual function and electrophysiology of rodents: the ALTEA-MICE project. Adv Space Res **33**: 1347–1351.
70. Narici L, *et al* (2004) The ALTEA/ALTEINO projects: studying functional effects of microgravity and cosmic radiation. Adv Space Res **33**: 1352–1357.
71. Tubiana M, *et al* (2009) The linear no-threshold relationship is inconsistent with radiation biologic and experimental data. Radiology **251**: 13–22.
72. Cucinotta FA, *et al* (2001) Space radiation and cataracts in astronauts. Radiat Res **156**: 460–466.
73. Blakely EA and Chang PY (2007) A review of ground-based heavy ion radiobiology relevant to space radiation risk assessment: cataracts and CNS effects. Adv Space Res **40**: 1307–1319.
74. Ainsbury EA, *et al* (2009) Radiation cataractogenesis: a review of recent studies. Radiat Res **172**: 1–9.
75. Shore RE, *et al* (2010) Epidemiological studies of cataract risk at low to moderate radiation doses: (not) seeing is believing. Radiat Res **174**: 889–894.
76. ICRP (1991) 1990 Recommendations of the International Commission on Radiological Protection. Publication ICRP N. 60. Annual ICRP Publications, Vol. 21. Pergamon Press; Oxford.
77. ICRP (2007) 2006 Recommendations 2006 of the International Commission on Radiological Protection. Publication ICRP N. 103. Annual ICRP Publications, Vol.37. Pergamon Press; Oxford.
78. Joubert A and Foray N (2007) [Intrinsic radiosensitivity and DNA double-strand breaks in human cells]. Cancer Radiother **11**: 129–142.
79. Joubert A, *et al* (2008) DNA double-strand break repair defects in syndromes associated with acute radiation response: at least two different assays to predict intrinsic radiosensitivity? Int J Radiat Biol **84**: 1–19.
80. Joiner MC, *et al* (2001) Low-dose hypersensitivity: current status and possible mechanisms. Int J Radiat Oncol Biol Phys **49**: 379–389.
81. Marples B, *et al* (2004) Low-dose hyper-radiosensitivity: a consequence of ineffective cell cycle arrest of radiation-damaged G2-phase cells. Radiat Res **161**: 247–255.
82. Skov KA, *et al* (1999) Radioresponsiveness at low doses: hyper-radiosensitivity and increased radioresistance in mammalian cells. Mutat Res **430**: 241–253.
83. Mitchell CR, *et al* (2002) Effects of exposure to low-dose-rate (60)co gamma rays on human tumor cells in vitro. Radiat Res **158**: 311–318.
84. Mitchell CR and Joiner MC (2002) Effect of subsequent acute-dose irradiation on cell survival in vitro following low dose-rate exposures. Int J Radiat Biol **78**: 981–990.
85. Vaganay-Juery S, *et al* (2000) Decreased DNA-PK activity in human cancer cells exhibiting hypersensitivity to low-dose irradiation. Br J Cancer **83**: 514–518.
86. Thomas C, *et al* (2008) Low-dose hyper-radiosensitivity of progressive and regressive cells isolated from a rat colon tumour: impact of DNA repair. Int J Radia Biol **84**: 533–548.
87. Slonina D, *et al* (2007) Low-dose radiation response of primary keratinocytes and fibroblasts from patients with cervix cancer. Radiat Res **167**: 251–259.
88. Foray N, *et al* (1997) Radiation-induced DNA double-strand breaks and the radiosensitivity of human cells: a closer look. Biochimie **79**: 567–575.
89. Foray N, *et al* (1998) Repair of radiation-induced DNA double-strand breaks in human fibroblasts is consistent with a continuous spectrum of repair probability. Int J Radiat Biol **74**: 551–560.
90. Foray N, *et al* (2005) The repair rate of radiation-induced DNA damage: a stochastic interpretation based on the

gamma function. J Theor Biol **236**: 448–458.
91. Chavaudra N, *et al* (2004) Quantified relationship between cellular radiosensitivity, DNA repair defects and chromatin relaxation: a study of 19 human tumour cell lines from different origin. Radiother Oncol **73**: 373–382.
92. Ponette V, *et al* (1996) Pulse exposure to ionizing radiation elicits rapid changes in cellular radiosensitivity. C R Acad Sci III **319**: 505–509.
93. Ponette V, *et al* (2000) Hyperfast, early cell response to ionizing radiation. Int J Radiat Biol **76**: 1233–1243.
94. Feinendegen LE (2005) Evidence for beneficial low level radiation effects and radiation hormesis. Br J Radiol **78**: 3–7.
95. Matsumoto H, *et al* (2009) A new paradigm in radioadaptive response developing from microbeam research. J Radiat Res (Tokyo) **50 Suppl A**: A67–A79.
96. Elmore E, *et al* (2008) Low doses of very low-dose-rate low-LET radiation suppress radiation-induced neoplastic transformation in vitro and induce an adaptive response. Radiat Res **169**: 311–318.
97. Carbone MC, *et al* (2009) The Cosmic Silence experiment: on the putative adaptive role of environmental ionizing radiation. Radiat Environ Biophys **48**: 189–196.
98. Ina Y, *et al* (2005) Suppression of thymic lymphoma induction by life-long low-dose-rate irradiation accompanied by immune activation in C57BL/6 mice. Radiat Res **163**: 153–158.
99. Lacoste-Collin L, *et al* (2007) Effect of continuous irradiation with a very low dose of gamma rays on life span and the immune system in SJL mice prone to B-cell lymphoma. Radiat Res **168**: 725–732.
100. Takahashi A, *et al* (2010) P53-dependent adaptive responses in human cells exposed to space radiation. Int J Radiat Oncol Biol Phys **78**: 1171–1176.
101. Regaud C and Lacassagne A (1927) Effets histophysiologiques des rayons de Roentgen et de Becquerel-Curie sur les tissus adultes normaux des animaux supérieurs. In: Regaud C eds. Radiophysiologie et Radiothérapie. pp. Presses Universitaires de France; Paris, France.
102. Bouchacourt ML (1911) Sur la différence de sensibilité aux rayons de Roentgen de la peau des différents sujets, et, sur le même sujet des différents régions du corps. In: Sciences. Splad eds. pp. 942–947. Masson; Paris.
103. Fertil B and Malaise EP (1981) Inherent cellular radiosensitivity as a basic concept for human tumor radiotherapy. Int J Radiat Oncol Biol Phys **7**: 621–629.
104. Deschavanne PJ and Fertil B (1996) A review of human cell radiosensitivity in vitro. Int J Radiat Oncol Biol Phys **34**: 251–266.
105. Joubert A and Foray N (2006) Repair of radiation-induced DNA double-strand breaks in human cells: History, Progress and Controversies. In: Landseer Br eds. In: New Research on DNA Repair. pp. Nova Science Publishers, Inc.
106. Swift M, *et al* (1991) Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med **325**: 1831–1836.
107. Hall EJ and Giacca AJ (2006) Radiobiology for the radiologist. Lippincott Wilkins & Williams; Philadelphia, USA.
108. Cucinotta F, *et al* (2001) Space radiation cancer risks and uncertainties for Mars missions. Radiat Res **156**: 682–688.
109. Cucinotta FA and Chappell LJ (2010) Non-targeted effects and the dose response for heavy ion tumor induction. Mutation Res **687**: 49–53.
110. De Ruyck K, *et al* (2006) TGFbeta1 polymorphisms and late clinical radiosensitivity in patients treated for gynecologic tumors. Int J Radiat Oncol Biol Phys **65**: 1240–1248.
111. Willems P, *et al* (2008) Polymorphisms in nonhomologous end-joining genes associated with breast cancer risk and chromosomal radiosensitivity. Genes Chromosomes Cancer **47**: 137–148.
112. Massart C, *et al* (2009) Prediction of the human radiosensitivity: what is the most relevant endpoint? Gene expressions, mutations or functions? In: Hauppauge Ny eds. Genotoxicity, Testing and Prediction. pp. Nova Science Publishers, Inc.; New York, USA.
113. Kushiro J, *et al* (1990) Absence of correlations between radiosensitivities of human T-lymphocytes in G0 and skin fibroblasts in log phase. Radiat Res **122**: 326–332.
114. Green MH, *et al* (1991) Comparative human cellular radiosensitivity: III. Gamma-radiation survival of cultured skin fibroblasts and resting T-lymphocytes from the peripheral blood of the same individual. Int J Radiat Biol **59**: 749–765.
115. Geara FB, *et al* (1992) Intrinsic radiosensitivity of normal human fibroblasts and lymphocytes after high- and low-dose-rate irradiation. Cancer Res **52**: 6348–6352.
116. Gooch PC and Berry CA (1969) Chromosome analyses of Gemini astronauts. Aerosp Med **40**: 610–614.
117. Yang TC, *et al* (1997) Biodosimetry results from space flight Mir-18. Radiat Res **148**: S17–S23.
118. Druzhinin SV (1999) [Cytogenetic effect in lymphocytes in astronauts after 2 lengthy flights on board MIR orbital station]. Aviakosm Ekolog Med **33**: 3–5.
119. Fedorenko B, *et al* (2001) Cytogenetic studies of blood lymphocytes from cosmonauts after long-term space flights on Mir station. Adv Space Res **27**: 355–359.
120. Fedorenko BS, *et al* (2000) [Cytogenetic studies of blood lymphocytes of cosmonauts after long-ter, space flights]. Radiats Biol Radioecol **40**: 596–602.
121. George K, *et al* (2001) Chromosome aberrations in the blood lymphocytes of astronauts after space flight. Radiat Res **156**: 731–738.
122. Wu H, *et al* (2001) Comparison of chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated in vitro with gamma rays. Phys Med **17 Suppl 1**: 229–231.
123. George K, *et al* (2003) In vivo and in vitro measurements of complex-type chromosomal exchanges induced by heavy ions. Adv Space Res **31**: 1525–1535.
124. George K, *et al* (2004) Chromosome aberrations of clonal origin are present in astronauts' blood lymphocytes. Cytogenet Genome Res **104**: 245–251.
125. Greco O, *et al* (2003) Biological dosimetry in Russian and Italian astronauts. Adv Space Res **31**: 1495–1503.
126. Durante M, *et al* (2003) Chromosome aberration dosimetry in cosmonauts after single or multiple space flights. Cytogenet Genome Res **103**: 40–46.
127. George K, *et al* (2005) Stability of chromosome aberrations

in the blood lymphocytes of astronauts measured after space flight by FISH chromosome painting. Radiat Res **164**: 474–480.

128. Horstmann M, *et al* (2005) Space radiation does not induce a significant increase of intrachromosomal exchanges in astronauts' lymphocytes. Radiat Environ Biophys **44**: 219–224.
129. Pelevina II, *et al* (2007) [Individual cytogenetic and molecular-biological characteristics of lymphocytes in blood of pilots and cosmonauts]. Radiats Biol Radioecol **47**: 141–150.
130. Fedorenko BS, *et al* (2008) [Cytogenetic effects on blood lymphocytes of cosmonauts after low doses of space radiation]. Aviakosm Ekolog Med **42**: 13–18.
131. George K, *et al* (2010) Persistence of space radiation induced cytogenetic damage in the blood lymphocytes of astronauts. Mutat Res 2010 Aug 14; **701**(1): 75–79.

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