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CYTOGENETIC STUDIES OF BLOOD (EXPERIMENT M111)

Lillian H. Lockhart, M.D.
The University of Texas Medical Branch, Galveston, Texas

ABSTRACT

The Skylab M111 experiment is a continuation of the preflight and post-flight chromosomal analyses of the flight crews that have been performed since the Gemini III mission. The experiment is designed to determine whether some space flight parameter produces cytogenetic effects in human cells and to provide biological radiation dosimetric capability in the event of significant radiation exposure to a flight crew.

On each of the Skylab flights, blood lymphocytes for analysis of chromosomes for structural defects were obtained from each of the prime crewmembers and from a ground-based control group before and after flight. The cultures were successful except for occasional specimens obtained on recovery day on board the recovery ship. These specimens had to be handled differently than the others. All specimens were in culture for 60 to 70 hours and then were processed by treatemnt with colcemid, a hypotonic solution, followed by fixation and flame-dry slide preparation. On each of the studies, two examiners each counted and analyzed 100 to 150 cells at random for structural defects of the chromosomes. Two types of defects were recorded. The minor defects included the following aberrations: chromatid fragments, chromosome fragments, and deletions. Structural rearrangements such as dicentrics, exchanges, ring chromosomes, and translocations were photographed, and the cells were karyotyped to delineate, when possible, the chromosome or chromosomes involved in the rearrangement.

Except for one study of a control subject, no individual study demonstrated greater than eight percent minor chromosomal structure defects. In considering the more involved structural rearrangements found in individuals, it is apparent that both the astronauts and the controls had one or more such defects on several occasions. These were found both before and after flight, but they were found more consistently in both groups after flight. This result seems to indicate that the flight itself was not a major contributing factor. The influence of repeated isotope injections given to astronauts and to controls must also be considered.

INTRODUCTION

It has been appreciated for some time that increased frequency of chromosomal aberration occurs in man following exposure to ionizing radiation. Information has been obtained by study of persons receiving an external body source, such as therapeutic dosage or of those accidently exposed. Others receiving radiation exposure from an internal source, such as the decay of radioisotopes administered for diagnosis or treatment, have also been analyzed. It is obvious that interpretation of such data may be fraught with many problems. The radiation exposure may be acute or chronic, partial or total body, repeated or a single event. The tissue studied and the time elapsed following exposure have also been quite variable.

Structural chromosomal aberrations are also known to occur following exposure to other environmental factors such as viruses (both DNA and RNA) acquired either through immunization or infection, to various chemicals such as benzene, and to numerous drugs.

Concern over the possible harm of low levels of radiation exposure centers mostly around its association with hereditary damage or malignancy. Essentially no information is available concerning radiation effects on the chromosomes of gonadal or meiotic cells of man and estimates of hereditary damage are based in large part on theoretical views. It should be remembered that we cannot extrapolate findings in somatic cells (in the case under discussion circulating lymphocytes) to gametic chromosomal patterns. On the other hand, concern regarding the cancer hazard in irradiated human populations has been suggested by well founded studies (1). A classic example is that of patients treated with x-rays for ankylosing spondylitis who have on the average a ten-fold increase in mortality from leukemia (2). These patients were reported by Buckton, *et al.* (3) in 1962 to have structural chromosomal damage of cultured peripheral leukocytes some years after the treatment. The fact that many agents which produce tumors in man and animals can also produce chromosomal aberrations in their cells is clearly established. This information coupled with the fact that in several rare human disorders (Bloom's syndrome, Fanconi's anemia and ataxia telangiectasia) there is a constitutional predilection for increased chromosomal aberrations as well as an increased incidence of leukemia and lymphoma has suggested that an increase in structural chromosome aberrations cannot be ignored.

These chromosomal aberrations are structural in nature, that is, they arise through breakage of the strands of chromatin. These breaks may occur either in one or in both chromatids of a single chromosome or multiple breaks may occur in several chromosomes within an individual cell. Following such accidents, the strands may or may not recombine within themselves or the broken ends of several chromosomes may

combine with each other. Two general types of aberrations occur depending on the stage of the cell cycle in which the break cccurs. If the cell is in the pre-DNA synthesis period, chromosome strands are single (chromatids) and if the accident occurs after synthesis, the chromosome consists of two chromatids. Chromosomes are technically examined in the metaphase stage of division because that is when they can be separated as individuals, so replication may or may not have occurred when we examine the chromosomes of peripheral lymphocytes, depending in part on the time in culture. In general, these two types of aberrations may be morphologically separated, however, in several instances it is impossible to tell whether the break occurred in the pre-DNA synthesis, and was replicated, or whether both strands were affected after replication. A break will produce a fragment that is generally lost in the next cell division.

Separation of the aberrations into chromatid or chromosome in nature is useful since the type of structural defect occurring in humans as a response to a specific exposure, has varied with the agent to which the person is exposed.

It is with these considerations in mind that the NASA program has wisely considered cytogenetic studies important in past years and has especially concentrated on such aspects in the Skylab program with extended missions and possible increase in radiation exposure.

MATERIALS AND METHODS

This discussion centers on experiments designed for Skylab 2 since results for the other missions are not yet complete. Blood lymphocyte studies were obtained on eleven occasions preflight and eight instances postflight from the three members of the crew, from three controls and from the backup crew until it was apparent that they would not replace the crew. The control group consisted of three persons in the NASA program who would have an environment somewhat similar to the crew over the experimental period except for the flight. A total of 90 cultures were processed and 79 have been analyzed, the remainder being specimens of the backup crew. Venous heparinized blood was drawn either at the Johnson Space Center or the Kennedy Space Center in one to two milliliters aliquots and was obtained at the time of drawing for other medical procedures. The cultures were instituted at the University of Texas Medical Branch on all occasions except for the first two postflight studies which were obtained aboard the recovery ship. Each sample was allowed to settle and five to seven drops of the buffy coat were placed in Chromosome Medium 1A. Four such cultures were initiated on each person from each blood drawing. The cultures were then incubated for a period of 60 to 70 hours at 37° C and processed by a

modified method of Moorehead (4). Colcemid was added to a concentration of 0.1μg/ml for two hours. The cell suspension was then treated with a hypotonic solution followed by numerous washings with fixative (3 methanol:1 acetic acid). Slides were prepared on the same day by flame drying and the cells stained with Wright's stain.

The slides were coded and each of two examiners studied from 100 to 150 cells on each specimen. Cells were selected on low magnification if they appeared to be analyzable and then examined under high magnification; each cell was counted and a search made for any type of structural defect. When a structural defect was found the cell was photographed for further analysis by karyotyping in an attempt to delineate whenever possible the chromosome and/or chromosomes involved in the aberrations.

The cells were scored for the following structural arrangements: chromatid and chromosome constrictions and gaps (not to be considered in this paper); chromatid and chromosome breaks, fragments, and deletions (to be referred to as minor defects); and dicentrics, rings, inversions, translocations, and exchanges (to be referred to as structural rearrangements).

RESULTS AND DISCUSSION

The results of the cyogenetic analysis of lymphocytes of the Skylab 2 astronauts are shown in table I. There were four studies that were unsuccessful. These involved the recovery day specimens of the crew and of one control. These cultures were instituted aboard ship and transported by portable incubator. Several of these specimens had a dark brown appearance upon arrival at the laboratory.

There were no individual studies from this mission that demonstrated greater than 8.0 percent minor structural defects except for one on specimen L, a control. The blood had been drawn aboard ship on the day before recovery. In only 16 studies did such aberrations appear in from 5.0 to 7.9 percent of the cells examined. In our laboratory under similar technical conditions where 13 000 cells a year are counted and analyzed, it is expected that three to four percent of the cells analyzed will show one or more breaks, deletions or fragments. In other laboratories with varying preparation of cells for study, this aberration incidence may even be slightly greater. These defects are known to increase in peripheral leukocyte cultures of persons following a viral illness such as measles or adenovirus, after administration of viral vaccines, after certain diagnostic x-ray studies, and after exposure to certain chemicals. This increase in response to such exposures is in general only temporary, and little can be suggested as to harmful effects.

TABLE I

Skylab 2

Date & Subject	No. Cells Examined	% Minor Defects	Structural Rearrangements
4-2-73 F-53	KSC		
C (26)	268	3.35	
K (43)	239	3.34	1 Exchange
W (34)	250	2.80	
A (80)	259	3.86	
H (55)	260	1.15	1 Inversion
L (14)	271	2.95	
Mc(84)	223	0.44	
M (65)	238	1.68	
S (72)	231	1.73	
4-24-73 F-31	KSC		
L (41)	277	1.44	
S (46)	244	2.45	
4-25-73 F-30	KSC		
A (28)	256	3.90	1 Exchange
H (85)	234	1.28	
L (38)	217	4.60	
4-26-73 F-29	KSC		
C (22)	273	1.83	1 Dicentric
K (12)	263	7.22	
W (59)	241	1.66	
A (7)	255	6.27	
H (89)	234	2.13	1 Dicentric
4-27-73 F-28	KSC		
C (31)	241	1.24	
K (10)	257	4.66	1 Translocation
W (69)	244	4.09	
5-1-73 F-24	JSC		
H (78)	242	0.83	
L (96)	247	4.05	2 Translocations
5-2-73 F-23	JSC		
C (64)	260	5.39	2 Rings, 1 Exchange
K (91)	257	3.89	
W (81)	250	5.20	
A (50)	244	6.56	

Crew: Conrad (C), Kerwin (K), Weitz (W)
Controls: Alexander (A), Hordinsky (H), La Pinta (L)
Backup Crew: McCandless (Mc), Musgrave (M), Schweickert (S)

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TABLE I (Continued)

Date & Subject	No. Cells Examined	% Minor Defects	Structural Rearrangements
5-7-73 F-18	JSC		
C (4)	247	3.64	
K (95)	230	3.48	1 Ring
W (13)	243	2.06	
A (29)	238	5.04	1 Dicentric 1 Exchange
5-8-73 F-17	JSC		
H (18)	257	0.78	
L (48)	267	3.74	
5-14-73 F-11	JSC		
C (66)	273	4.03	1 Exchange
K (35)	246	4.47	1 Dicentric 1 Exchange
W (51)	243	5.34	
A (25)	244	7.37	1 Exchange
H (5)	245	2.85	
L (17)	277	1.80	1 Dicentric
5-24-73 F-1	KSC		
C (47)	269	1.86	
K (77)	231	4.76	
W (99)	239	5.43	
A (53)	238	3.78	1 Exchange
H (83)	241	2.90	
L (98)	259	3.47	
Flight= 5-25-73			
6-21-73 R-1	Ship		
A (92)	258	7.75	1 Dicentric
H (67)	Unsuccessful		
L (97)	139	11.51	
6-22-73 R+0	Ship		
C (15)	Unsuccessful		
K (90)	Unsuccessful		
W (56)	Unsuccessful		

TABLE I (Concluded)

Date & Subject	No. Cells Examined	% Minor Defects	Structural Rearrangements
6-23-73 R+1	Ship		
C (23)	257	3.50	
K (32)	234	4.70	1 Chromatid Exchange
W (45)	255	1.57	
A (93)	274	5.11	
H (76)	266	2.25	
L (86)	249	4.01	
6-26-73 R+4	JSC		
C (19)	261	4.21	1 Tricentric 1 Exchange
K (63)	264	2.65	1 Ring
W (57)	290	3.10	1 Dicentric, 1 Ring
A (94)	256	2.73	
H (71)	234	2.99	2 Dicentrics
L (82)	250	2.00	
6-29-73 R+7	JSC		
C (27)	245	1.22	
K (44)	260	6.15	1 Dicentric
W (70)	248	2.02	1 Dicentric
A (87)	239	6.28	2 Exchanges 1 Dicentric
H (3)	232	0.43	
L (52)	248	2.82	1 Dicentric 1 Exchange
7-5-73 R+13	JSC		
C (88)	223	6.72	1 Exchange
K (60)	260	4.62	
W (54)	244	4.91	
A (37)	273	6.23	1 Dicentric 3 Exchanges
7-9-73 R+17	JSC		
K (30)	276	2.90	
7-10-73 R+18	JSC		
C (68)	256	7.03	1 Tricentric
W (73)	246	4.07	1 Dicentric
A (49)	262	3.81	1 Ring
H (33)	255	0.78	
L (42)	238	2.10	

Table II lists the radioisotope injections administered to crew and controls alike in the present study. You will note that only one blood culture was instituted prior to such administration (4-2-74), and no one in the crew, backup crew, or control group had greater than 3.86 percent aberrations on the first study. This may well be chance because at various other occasions throughout the experiment, each person demonstrated such low values. It is quite possible that control L had a viremia at the time in which the 11.51 percent aberrations were found, and throughout the remainder of the study his values returned to expected levels.

TABLE II. ISOTOPE INJECTIONS

1973

4/2	4/24	5/2	5/7	5/14	5/23	6/21	6/22	7/5	8/2	8/28
	^{125}I					^{125}I		^{125}I	^{125}I	^{125}I
	^{51}Cr					^{51}Cr		^{51}Cr	^{51}Cr	^{51}Cr
	^{35}S					^{35}S		^{35}S		^{35}S
	^{3}H				^{3}H	^{3}H		^{3}H	^{3}H	^{3}H
^{14}C										
	^{42}K	^{42}K	^{42}K	^{42}K	^{42}K		^{42}K	^{42}K		
						^{59}Fe				

Speculation as to the significance of structural rearrangements is more difficult. It is noteworthy that in the first culture there was one crewmember and one control with evidence of breakage and recombination. This is not characteristic of the general population. It has been reported that such aberrations as dicentrics, rings, inversions, and exhanges occur very rarely, (fig. 1). Bloom, *et al.* (5) found only one dicentric and no rings in 7188 cells examined. Bender, *et al.* (6) reported 3 dicentrics and no ring chromosomes in 1642 cells from normal, unirradiated individuals. In our experience, it is less common. We would be the first to admit, however, that neither the crew nor the control group under discussion today are members of the general population. As to the crew, we might speculate for a moment what possible exposures occurred prior to chromosome culture. Obviously in such a professional lifetime there are many, varied experiences in comparison to the general population. Perhaps they came to Skylab and even to

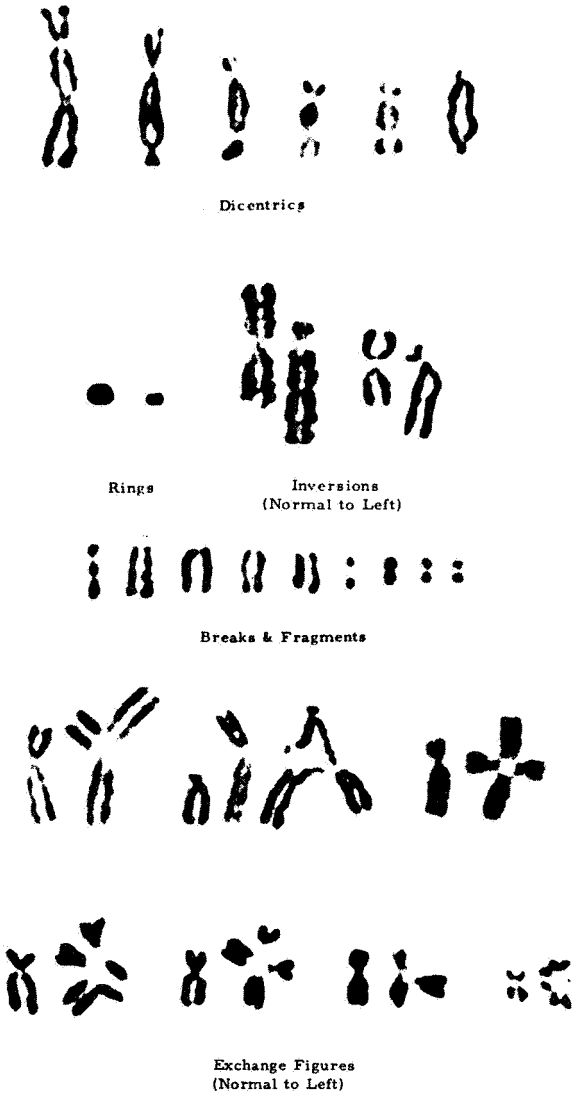

Figure 1. Skylab abnormalities.

NASA with these findings. Gooch and Berry (7) reporting on the chromosome aberrations of the Gemini astronauts also noted an occasional dicentric or ring chromosome.

In reviewing the medical log of this mission, one wonders about such potential problems as exposure to various gases, too high temperatures, to the atmospheric conditions in flight, and in fact, to weightlessness. Prince, *et al.* (8) reported observations on man in an oxygen-helium environment and included chromosome study. They noted up to 4 percent chromatid-type lesions in the subjects. There is otherwise no information relating such variables to chromosome aberration. There is very good documentation regarding illness and drug ingestion in the astronauts of Skylab 2 and comparison of this data with the chromosomal pattern does not suggest a cause and effect relationship.

The appearance of one or more structural rearrangements in 250 cells is unusual, however, in this study there is no difference in the crew and in the control group in regard to such aberrations as they occur sporadically throughout. However, there is one factor common to both groups that cannot be ruled out and this is the administration of radioisotopes for various medical studies. Some subjects from both groups have had such a series on repeated occasions even prior to this mission.

It is indeed impossible to say that the appearance of such aberrations increased in the crew following the mission, and frustrating to admit that what might have been the most important specimens from the crew were unsuccessful. Initially it was thought culture failure may have been the result of transporting the specimens and of difficult culture conditions. In personal communication with Dr. S. E. Ritzman (experiment M112), however, it was realized that the problem may be related to defective lymphocyte transformation and/or DNA synthesis on the day of recovery.

There are several obvious problems in this experiment. Primarily there is no normal control group, that is persons from the general population not receiving isotopes and without previous high altitude or orbital flight experience, analyzed simultaneously. Secondly, we did not have occasion to study the crew over a long period prior to introducing other variables. Arrangements have been made, however, to continue to study them intermittently to determine whether these findings are only temporary. Thirdly, in planning the culture time, a shorter period would have allowed for study of more cells after only one division in culture, and perhaps finding an even greater number of aberrations.

SUMMARY

In summary, I would like to reiterate that the appearance of structural rearrangement in 250 cells examined from one specimen is unusual, that it may only be a temporary finding, and that the data does not seem to suggest that the external sources of radiation to which the crew were exposed in orbit resulted in an aberration increase.

REFERENCES

1. Court Brown, W. M., K. E. Buckton and A. S. McLean. 1965. Quantative Studies of Chromosome Aberrations in Man Following Acute and Chronic Exposure to X-rays and Gamma Rays. *Lancet* I: 1239-1241.

2. Bender, Michael A. 1969. Human Radiation Cytogenetics. *Advanced Radiation Biology* 3: 215-275.

3. Buckton, K. E., P. A. Jacobs, W. M. Court Brown and R. Doll. 1962. A Study of the Chromosome Damage Persisting After X-ray Therapy for Ankylosing Spondylitis. *Lancet* II: 676-682.

4. Moorehead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford. 1960. Chromosome Preparations of Leukocyte Cultures from Peripheral Blood. *Experimental Cell Research* 20: 613.

5. Bloom, A. D., S. Neriishi, A. A. Awa, T. Honda and P. G. Archer. 1967. Chromosome Aberrations in Leucocytes of Older Survivors of the Atomic Bombings of Hiroshima and Nagasaki. *Lancet* II: 802-805.

6. Bender, M. A. and P. C. Gooch. 1966. Somatic Chromosome Aberrations Induced by Human Whole-Body Irradiation: The "Recuplex" Criticality Accident. *Radiation Research* 29:568-582.

7. Gooch, P. C. and C. A. Berry. 1969. Chromosome Analyses of Gemini Astronauts. *Aerospace Medicine* 40: 610-614.

8. Prince, J. E., L. H. Mori, H. J. Zeft, B. E. Welch and A. P. Amarose. 1968. Observations on Man in an Oxygen-Helium Environment at 380 mm Hg Total Pressure: V Chromosome Profiles. *Aerospace Medicine* 39: 111-114.