N 69 21149
NASA CR 100458

NAMI-1056

THE SOMATIC CHROMOSOMES OF THE MONGOLIAN GERBIL (Meriones unguiculatus)

Steven P. Pakes

CASE FILE
COPY

JOINT REPORT

NAVAL AEROSPACE MEDICAL INSTITUTE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

January 1969

This document has been approved for public release and sale;
its distribution is unlimited.

This document has been approved for public release and sale;
its distribution is unlimited.

THE SOMATIC CHROMOSOMES OF THE MONGOLIAN GERBIL (Meriones unguiculatus)*

Steven P. Pakes

Bureau of Medicine and Surgery
MR005.04-0032.4

NASA Order W12,766

Approved by

Ashton Graybiel, M.D.
Head, Research Department

Released by

Captain J. W. Weaver, MC USN
Commanding Officer

3 January 1969

*This research was conducted under the sponsorship of the Office of Space Science and Applications, Bioscience Programs, Environmental Biology, National Aeronautics and Space Administration.

NAVAL AEROSPACE MEDICAL INSTITUTE
NAVAL AEROSPACE MEDICAL CENTER
PENSACOLA, FLORIDA 32512

SUMMARY PAGE*

THE PROBLEM

The present study was initiated to characterize the somatic chromosomes of the Mongolian gerbil (Meriones unguiculatus) prior to conducting experiments concerned with the effects of various environmental factors encountered in space flight on mammalian chromosomes.

FINDINGS

From the study of bone marrow cells after intraperitoneal injection of colchicine it was determined that the diploid number of chromosomes for the Mongolian gerbil is 44.

The karyotype was constructed by arranging the chromosomes into four distinct groups and includes 32 meta- or submetacentric and 10 acrocentric autosomal chromosomes. The X element was identified as a large submetacentric chromosome and the Y element as a medium-sized submetacentric chromosome.

ACKNOWLEDGMENTS

The advice and help of Miss Glenda Cowart are greatly appreciated, as is the technical assistance of Mrs. Sheila Ward and Mr. Larry Moorman. The help of Robert Barrett in preparing the photographs is also gratefully acknowledged.

---

*The animals used in this study were handled in accordance with the 'Principles of Laboratory Animal Care' established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

INTRODUCTION

The Mongolian gerbil (Meriones unguiculatus), Milne-Edwards, 1867, is a desert rodent, native of Mongolia and northeastern China. It is classified in the order Rodentia, family Cricetidae, and subfamily Gerbillinae, of which Meriones is one of ten genera (11).

Many animals in the subfamily Gerbillinae are being investigated as possible experimental models in various biological studies, but recently the Mongolian gerbil has evolved as the most commonly utilized and extensively studied. Schwentker (10) has enumerated the many characteristics of the gerbil that make it potentially suitable as a laboratory animal. Rich's review (7) of the subject points out that the Mongolian gerbil has been used in many areas of research, including behavioral psychology, pharmacology, neurology, endocrinology, oncology, cholesterol metabolism, parasitology, and infectious disease studies.

Our laboratory is interested in the possible application of these animals in studies concerned with the biological effects of experimental factors experienced during prolonged space flight. Physiological characteristics of the gerbil which make it suitable for such studies include a high degree of heat tolerance and of temperature regulation (8) and a relatively high resistance to irradiation (2). In addition, it thrives on a variety of diets, requires little or no additional water to that in the diet, urinates only a few drops per day, and voids practically dry feces. The gerbil also is relatively free of naturally occurring viral, bacterial, and parasitic diseases.

The present study was initiated to characterize the somatic chromosomes of this laboratory animal, preliminary to studies concerned with the effects of various space environments on mammalian chromosomes. Other descriptions of the chromosomes of this species are contained in reports by Awa et al. (1) and by Nadler and Lay (6).

PROCEDURE

ANIMALS

Five male and five female Mongolian gerbils (as shown in the Appendix), three months to one year old, were used in this study. They were from a closed, but not inbred, colony derived from gerbils purchased from Tumblebrook Farm, Inc., Brant Lake, New York, in 1960. All Mongolian gerbils in this country have probably originated from this source. The colony at our laboratory is housed on ground corncob bedding in polypropylene cages measuring 15" x 13" x 6 3/4". The environment is controlled at $74^{\circ} \pm 2^{\circ}$ F, and 50% ± 5% relative humidty, with a 14-hour light period. The gerbils are provided Purine Laboratory Chow and water ad libitum.

CHROMOSOME PREPARATIONS

Chromosome preparations were made directly from bone marrow cells using the technique of Ford and Hamerton (3), modified as follows:

1) Gerbils were injected intraperitoneally with 0.5 ml of 0.03% aqueous solution of colchicine. The animals were killed with chloroform two to three hours later. Both femurs and tibias were removed and the epiphyses amputated. The marrow was aspirated into a syringe coated with heparin (1000 units/ml) with a 22-gauge needle.

2) The bone marrow sample was flushed into a 15-ml conical centrifuge tube containing Hanks' balanced salt solution prewarmed to 37° C, and was washed by gentle agitation with a pipette. The sample was centrifuged for five minutes at 100 g, and the supernatent decanted.

3) The cells were swollen by suspending them in prewarmed 0.9% sodium citrate solution and by incubation at 37° C for 20 minutes. The sample was centifuged for five minutes at 36 g and the supernatent decanted.

4) Fresh methanol and glacial acetic acid (3:1) fixative, precooled to 4° C, was gently added to the cell button. After 30 minutes' fixation at 4° C the sample was again centrifuged at 36 g for five minutes, the supernatent decanted, and fresh fixative added for 15 minutes; this step was repeated twice. Finally, the cells were gently agitated in enough fresh fixative to yield a moderately dense cell suspension.

5) One or two drops of cell suspension were placed on clean slides that had been dipped in iced distilled water, and the mixture was ignited to hasten drying and enhance the spreading of chromosomes (9).

6) The chromosome preparations were stained with 2% acetic orcein for one hour, dehydrated in increasing concentrations of ethyl alcohol, cleared in xylene, and coverslipped by using Permount (Fisher Scientific Company).

ANALYSIS

Slides were scanned at 100 X magnification for suitable metaphase plates. Criteria for suitability included: 1) Metaphase plates must appear intact; 2) metaphase plates must be distinctly identifiable; 3) each chromosome must be identified as a distinct morphological entity with a minimum of overlapping.

A cell selected for final counting was included in the analysis for that animal. Counting was done either by direct microscopy with a hand counter or from photographs of the metaphases. Direct counts through the microscope were made at least three times for each cell.

Photomicrographs were taken on 35-mm Kodak high-contrast copy film and a Wratten 58 green filter was used for increasing contrast. Selected metaphase plates were enlarged to approximately 2750 X for study of chromosome morphylogy. Ten male and ten female cells were studied to construct the karyotype for each sex.

RESULTS

The chromosome counts made from 743 bone marrow cells of five male and five female Mongolian gerbils are shown in Table I. A total of 675 cells (91%) had a diploid number of 44.

Table I

Chromosome Counts of Mongolian Gerbil Bone Marrow Cells

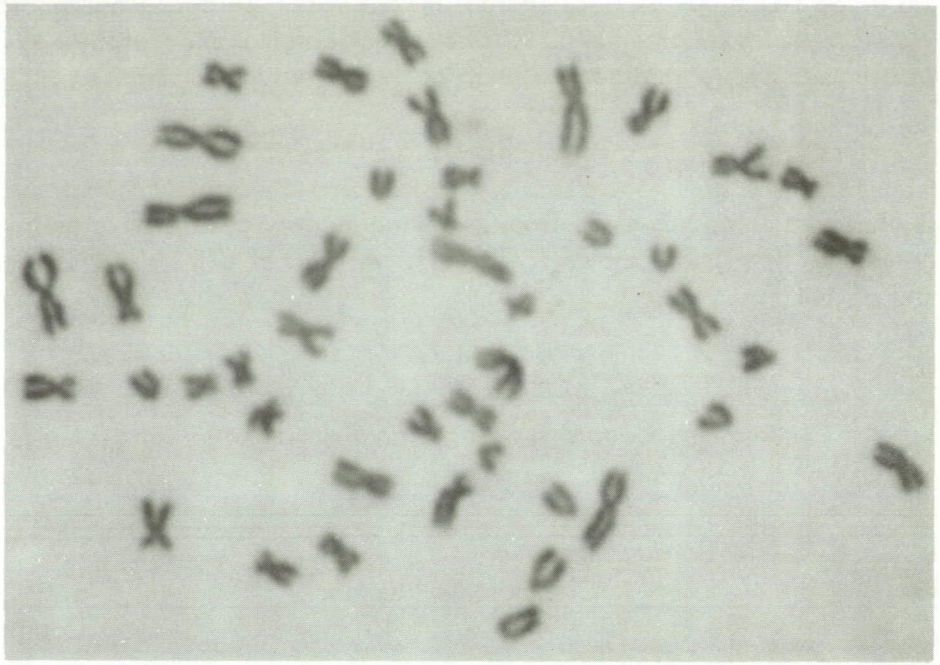
Sex	Chromosome Number					Total Cells Counted
	<43	43	44	45	>45	
M	-	7	91	1	-	99
M	-	2	111	-	-	113
M	-	-	52	4	1	57
M	1	3	51	-	-	55
M	1	5	63	4	1	74
F	-	5	70	1	-	76
F	-	2	66	-	-	68
F	-	4	57	-	1	62
F	3	5	53	2	-	63
F	2	6	61	7	-	76
Totals	7	39	675	19	3	743

Karyotypes were prepared by arranging the chromosomes into the following groups according to size and centromeric position.

I. Large metacentric and submetacentric chromosomes.
II. Small and medium metacentric and submetracentric chromosomes.
III. Near subtelocentric chromosomes.
IV. Acrocentric chromosomes.

Figures 1 and 2 depict metaphase plates and representative karyotypes for male and female Mongolian gerbils, respectively. Homologous chromosomes in some instances

a)



b)

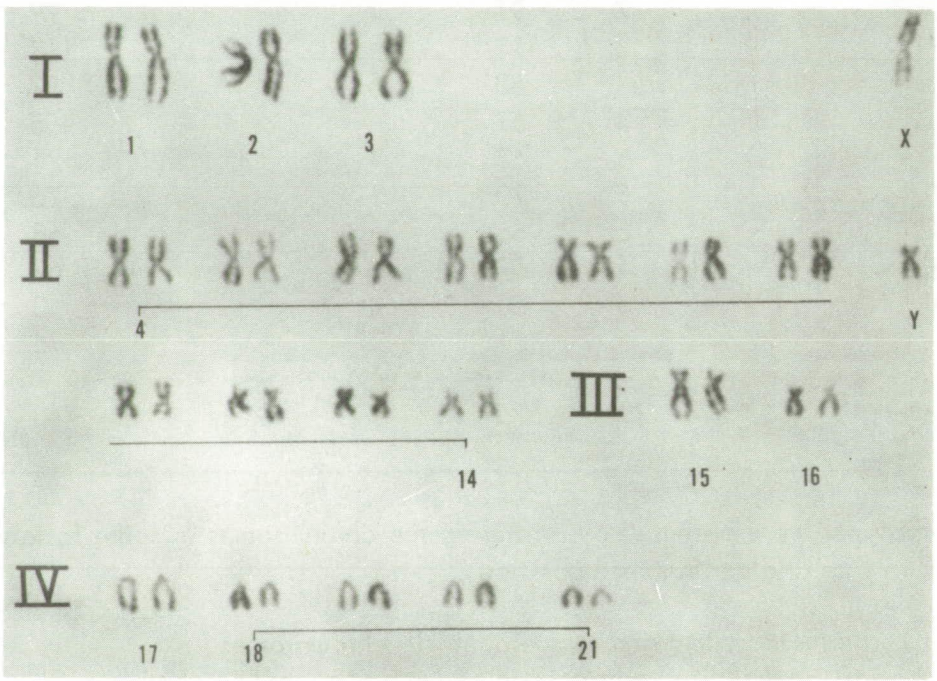
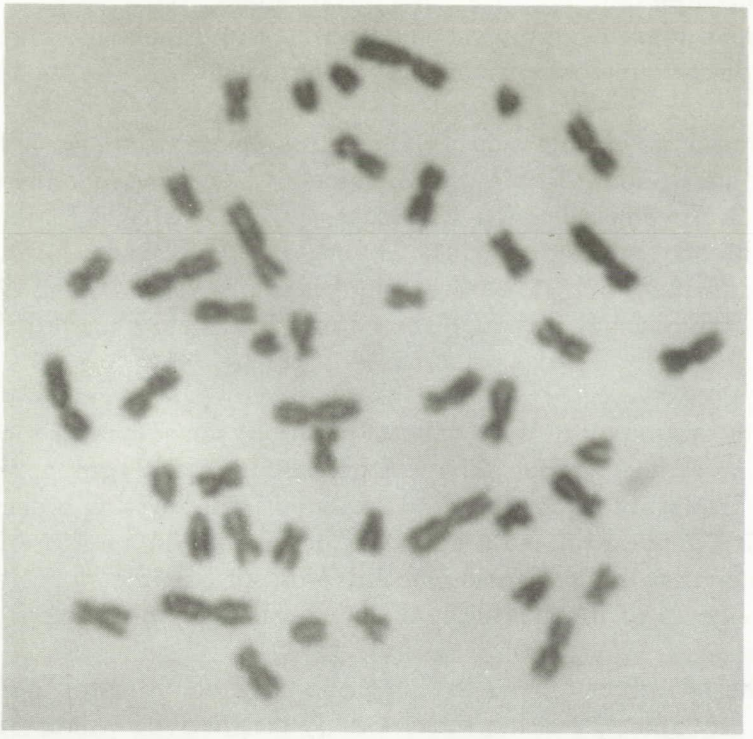


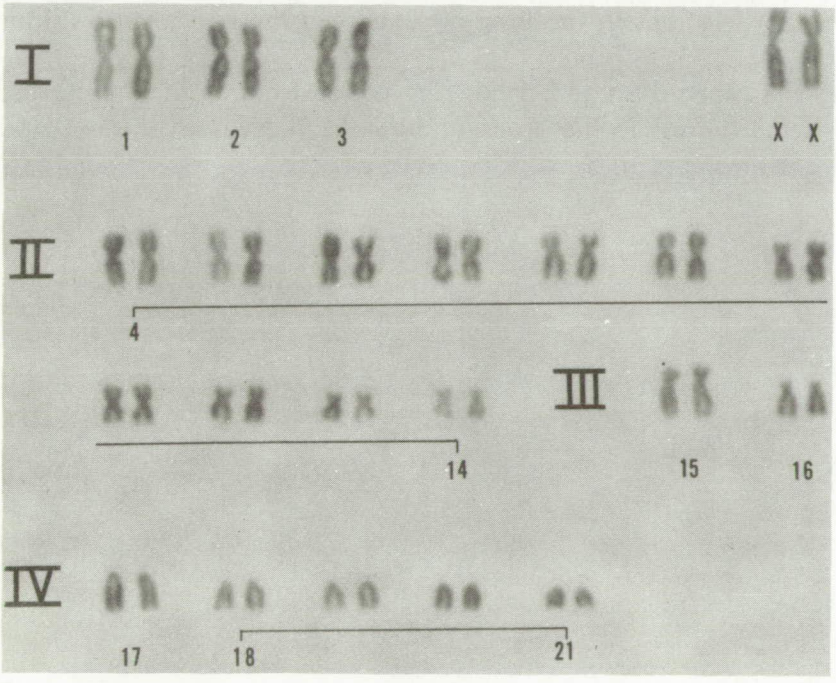
Figure 1

Chromosome complement of male Mongolian gerbil bone marrow cell.

a) Metaphase plate
b) Karyotype. Acetic orcein stain, reproduced at 1250 X.



a)



b)

Figure 2

Chromosome complement of female Mongolian gerbil bone marrow cell.

a) Metaphase plate
b) Karyotype. Acetic orcein stain, reproduced at 1000 X.

could be accurately paired and identified and are numbered separately. In other instances, however, such identification was impossible, and presumable chromosome pairs were arranged in order of decreasing size within a group.

Group I - Three pairs of autosomes and the X chromosome are included in this group and are individually identifiable. Chromosome #1 is submetacentric and is the largest chromosome in the complement. Chromosome #2 is nearly as large as #1 in most preparations and is clearly submetacentric. Chromosome #3 is metacentric and is the smallest chromosome in the group. The X element is usually slightly smaller than #2 and is a submetacentric chromosome, although the centromere is more medial than in chromosome #1 and #2.

Group II - This group contains 11 pairs of meta- or submetacentric chromosomes. Positive identification of homologous pairs was impossible for this group; therefore, pairs were matched as closely as possible and arranged in decreasing order of size. The chromosome remaining unpaired in male cells was consistently a medium-sized submetacentric and was designated the Y chromosome. It was not possible to positively identify the Y chromosome because of the similarity of the members of this group.

Group III - The two chromosomes in this group have centromeres located more subtelocentric than any others, making them notably distinctive. Chromosome #15 is easily distinguishable from #16 because of its larger size. Also, in many preparations #15 has secondary constrictions in the long arms next to the centromere (Figure 3).

Group IV - The Mongolian gerbil has five pairs of acrocentric chromosomes. Chromosome #17 is the largest in the group and can be individually identified. Chromosomes #18 to #21 are arranged in decreasing order of size.

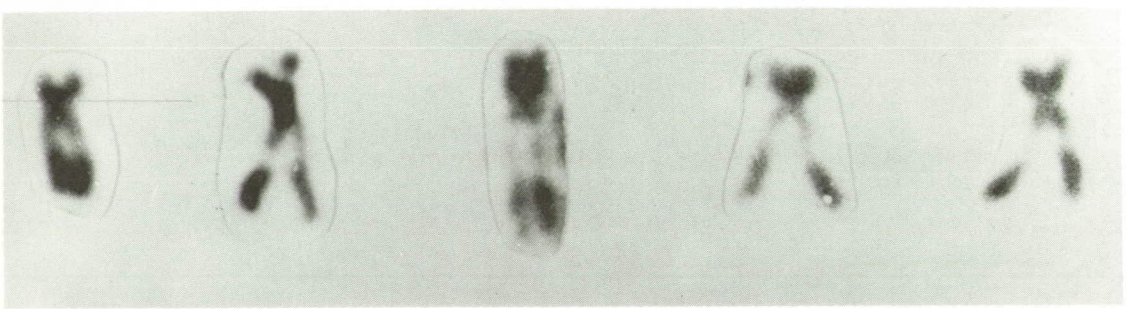


Figure 3

Chromosome #15 from different preparations showing secondary constrictions in long arms.

DISCUSSION

Previous attempts in culturing peripheral blood lymphocytes from Mongolian gerbils for chromosome studies were unsuccessful (unpublished data); therefore, direct examination of bone marrow cells after intraperitoneal injection of colchicine was performed. It is felt that the high concentration of serum lipids in the Mongolian gerbil had interfered with the culture of lymphocytes.

The diploid number of 44 for M. unguiculatus determined in the present study is in agreement with that observed in cultured spleen and liver cells by Awa et al. (1) and in direct preparation of bone marrow cells by Nadler and Lay (6). The minimal deviation from the mode shown in Table I of this study was primarily negatively skewed, involved random chromosomes, and was, therefore, considered technique artifact.

In the report of Awa and coworkers (1) it was difficult to discern exact chromosome morphology from their figures, but there appear to be few differences from the present study in the autosomal complement. They described the sex chromosomes as a medium-sized subterminal X, and the Y element as the smallest chromosome in the complement, having a terminally located centromere. This differs from the large submetacentric X and medium-sized submetacentric Y chromosomes described in this study. Karyotypic disparities from early cytogenetic studies are not uncommon and can usually be attributed to differences in technique.

The karyotype proposed by Nadler and Lay (6) is in essential agreement with that presented here. The method of grouping is different, but both studies identify 32 meta- or submetacentric and 10 acrocentric autosomal chromosomes, and a large submetacentric X element. The only difference exists in the designation of the Y chromosome. The Y chromosome in their karyotype has a more submedially located centromere, is larger than that described in this report, and appears to have secondary constrictions of the long arms, closely resembling chromosome #15 (Figure 3) of this study. An explanation for this discrepancy may be that intraspecific Y chromosome polymorphism exists in the Mongolian gerbil, as has been described in Syrian hamsters (5) and in some strains of laboratory rats (4). Chromosome studies of other Mongolian gerbil colonies may help to elucidate this matter. Presently, the significance of intraspecific sex chromosome polymorphism or remodeling is not understood.

REFERENCES

1. Awa, A., Sasaki, M., and Takayama, S., An in vitro study of the somatic chromosomes in several mammals. Jap. J. Zool., 12:257-265, 1959.

2. Chang, M. C., Hunt, D. M., and Turbyfill, C., High resistance of Mongolian gerbils to irradiation. Nature, 203:536-537, 1964.

3. Ford, C. E., and Hamerton, J. L., A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Tech., 31:247-251, 1956.

4. Hungerford, D. A., and Nowell, P. C., Sex chromosome polymorphism and the normal karyotype in three strains of the laboratory rat. J. Morph., 113:275-286, 1963.

5. Lehman, J. M., Macpherson, I., and Moorhead, P. S., Karyotype of the Syrian hamster. J. Nat. Cancer Inst., 31:639-650, 1963.

6. Nadler, C. F., and Lay, D. M., Chromosomes of some species of Meriones (Mammalia: Rodentia). Zeitschr. Säugetierk., 32:285-291, 1967.

7. Rich, S. T., The Mongolian gerbil (Meriones unguiculatus) in research. Lab. Animal Care, 18:235-243, 1968.

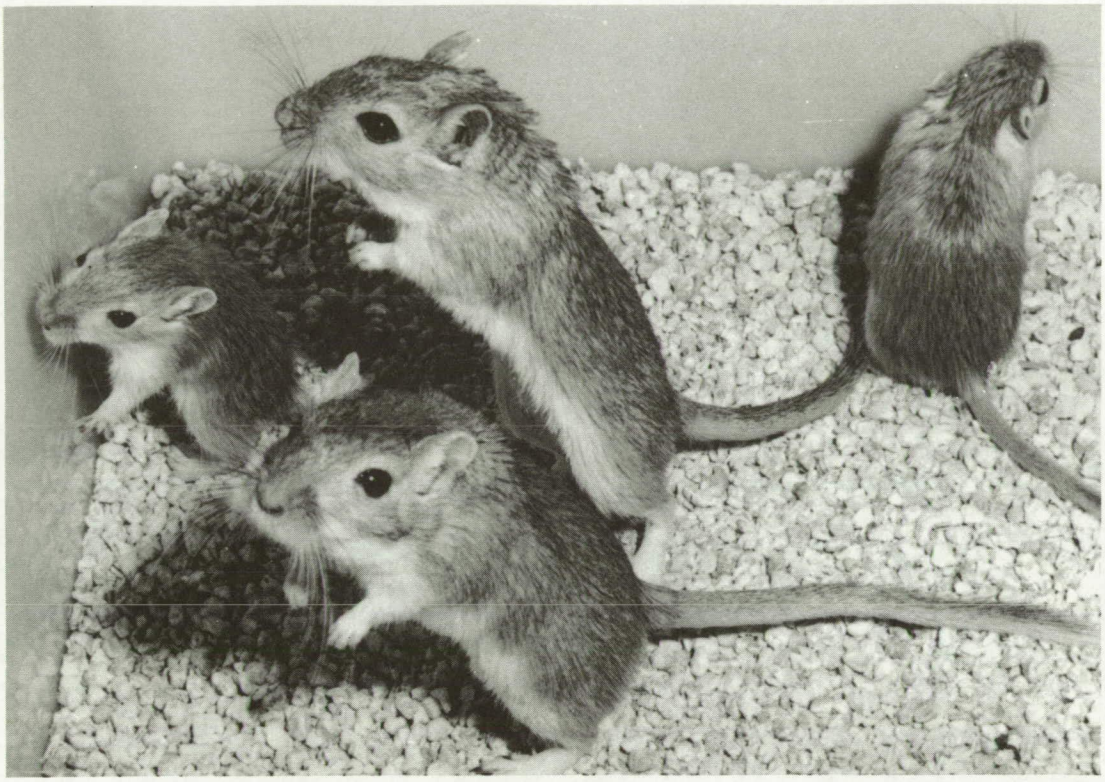
8. Robinson, P. F., Metabolism of the gerbil, Meriones unguiculatus. Science, 130: 502-503, 1959.

9. Scherz, R. G., Blaze drying, by igniting the fixative, for improved spreads of chromosomes in leucocytes. Stain Tech., 37:386, 1962.

10. Schwentker, V., The gerbil, a new laboratory animal. Illinois Vet., 6:5-9, 1963.

11. Simpson, G. G., The principles of classification and a classification of mammals. Bull. Am. Museum Nat. Hist., 85:88, 1945.

Appendix A



Male and female Mongolian gerbils with two of their progeny.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*

Naval Aerospace Medical Institute
Pensacola, Florida 32512

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

N/A

3. REPORT TITLE

THE SOMATIC CHROMOSOMES OF THE MONGOLIAN GERBIL (Meriones unguiculatus)

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

N/A

5. AUTHOR(S) *(First name, middle initial, last name)*

Steven P. Pakes, MAJOR, VC USA

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
3 January 1969	10	11

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
NASA Order W12,766	
b. PROJECT NO.	NAMI-1056
c. BuMed MR005.04-0032	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	4

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
N/A	N/A

13. ABSTRACT

This study was initiated to characterize the somatic chromosomes of the Mongolian gerbil (Meriones unguiculatus) prior to conducting experiments concerned with the effects of various environmental factors encountered in space flight on mammalian chromosomes.

From the study of bone marrow cells after intraperitoneal injection of colchicine it was determined that the diploid number of chromosomes for the Mongolian gerbil is 44.

The karyotype was constructed by arranging the chromosomes into four distinct groups and includes 32 meta- or submetacentric and 10 acrocentric autosomal chromosomes. The X element was identified as a large submetacentric chromosome and the Y element as a medium-sized submetacentric chromosome.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Somatic chromosomes						
Mongolian gerbil						
Meriones unguiculatus						
Cytogenetics						
Laboratory animals						