DETAILED DESCRIPTION AND NOMENCLATURE OF HIGH RESOLUTION G-BANDED HORSE CHROMOSOMES AND CYTOGENETIC ANALYSIS OF HEREDITARY MULTIPLE EXOSTOSIS IN HORSES

by

Alma Maciulis

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE in

Animal, Dairy and Veterinary Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah
1984
ACKNOWLEDGEMENTS

I wish to thank Dr. Thomas D. Bunch for his assistance and support in this project.

I also wish to express my sincere appreciation to the remaining members of my committee, Dr. J.L. Shupe, Dr. N.C. Leone, and Dr. E.J. Gardner for their assistance, time and knowledge contributed to this project. Also to Dr. W.C. Foote, who sat in on my defense, my deepest gratitude for his time and assistance.

Special thanks to Dean Stock, Chad Smith, and Cole Evans for their invaluable assistance.

To my adopted parents, Al and Tamara Maciulis, all my love for their support. Without them, this work would never have begun.

Mostly, my love and gratitude to my husband, Algirdas, and daughters, Laura and Irene, whose love and patience made this goal possible.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>Cytogenetics of the Domestic Horse</td>
<td>4</td>
</tr>
<tr>
<td>Hereditary Multiple Exostosis</td>
<td>6</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>RESULTS</td>
<td>13</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>33</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>37</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Technique used for obtaining high resolution elongated horse chromosomes.</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>Diagrammatic representation of horse chromosome 1.</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>Proposed standard domestic horse G-band karyotype.</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>High resolution G-band idiogram.</td>
<td>23</td>
</tr>
<tr>
<td>5.</td>
<td>Metaphase spread showing 1-31 association.</td>
<td>24</td>
</tr>
<tr>
<td>6.</td>
<td>Karyotype prepared from 1-31 association.</td>
<td>25</td>
</tr>
<tr>
<td>7.</td>
<td>Control male horse NORs.</td>
<td>26</td>
</tr>
<tr>
<td>8.</td>
<td>Control male horse C-bands.</td>
<td>27</td>
</tr>
<tr>
<td>9.</td>
<td>Exostosis female horse NORs.</td>
<td>28</td>
</tr>
<tr>
<td>10.</td>
<td>Exostosis male horse NORs.</td>
<td>29</td>
</tr>
<tr>
<td>11.</td>
<td>Exostosis female horse C-bands.</td>
<td>30</td>
</tr>
<tr>
<td>12.</td>
<td>Exostosis male horse C-bands.</td>
<td>31</td>
</tr>
<tr>
<td>13.</td>
<td>G-band comparison between control and exostosis horse chromosomes.</td>
<td>32</td>
</tr>
</tbody>
</table>
ABSTRACT

Detailed Description and Nomenclature of High Resolution G-banded Horse Chromosomes and Cytogenetic Analysis of Hereditary Multiple Exostosis in Horses

by

Alma Maciulis, Master of Science
Utah State University, 1984

Major Professor: Dr. Thomas D. Bunch
Department: Animal, Dairy & Veterinary Sciences

A reliable technique for obtaining late prophase, prometaphase and greater numbers of metaphase chromosomes is presented. The increased number of mitoses and high resolution chromosomes were obtained using a modification of the typical three-day blood culture technique in which the cell cycle was temporarily blocked at the G_1–S border with methotrexate. A high resolution G-band idiogram is presented using a system similar to the standardization of human chromosomes. The use of this technique and system of nomenclature will facilitate routine analyses of horse chromosomes and provide a basis for the identification and recording of chromosome aberrations in this species. C-bands and NORs of the domestic horse are also described.
C-bands, NORs and high resolution G-bands of control horses were compared with those of horses affected with Hereditary Multiple Exostosis. No differences were found on NORs, C- and G-bands between control and exostosis horses.

(39 pages)
INTRODUCTION

Studies on Hereditary Multiple Exostosis (HME) at Utah State University began in 1968 when a five-year-old thoroughbred male horse was brought to the Veterinary Science Department for diagnosis. It was determined that the horse had HME.

Hereditary Multiple Exostosis is a skeletal disease which mainly affects the long bones of the body with no tumors ever discovered on the skull. It has been recognized in horses, man, dogs, cats and several other animal species.

Selected matings of the affected male horse with 22 clinically normal female horses produced 19 colts for study, 7 nonaffected and 12 with bony tumors. Pedigrees prepared from the thoroughbred's ancestors and offspring showed that a dominant autosomal gene was involved. Offspring have a 50% chance of inheriting HME from a heterozygous parent, and the risk is even greater when both parents are affected. Cytogenetic analysis (which utilized older and less sophisticated techniques) of affected animals showed no characteristic chromosome abnormalities.

Pedigrees were also prepared from six human families
with members showing clinical signs of HME. The pattern of inheritance was also that of a single dominant autosomal gene. Prepared karyotypes had no diagnostic anomalies. The horse has been used as a model for the study of HME in humans. The studies at Utah State University have shown many similarities between HME affected horses and humans. Cytogenetic, radiological, histological and etiological patterns are similar in both species, although horses are not known to develop malignant tumors as sometimes occurs in man. At present, the original affected male horse 21 years old, is being kept for observation to determine whether a malignancy will occur.

Further cytogenetic studies utilizing high-resolution chromosome banding techniques were initiated to determine whether a marker could be identified on one or more chromosomes of horses affected with HME. The contemporary G-band horse standard karyotype contained too few bands to allow for an adequately detailed comparison of chromosome linear structure. Based on previous cytogenetic studies of HME affected animals, it was apparent that only high resolution G-banding techniques would allow for sufficient numbers of bands per chromosome for a definitive comparative study. Therefore, the first objective was to prepare a standard high-resolution
G-banded horse karyotype for use in comparative studies, which would facilitate the detection of small changes in chromosome structure. The second objective of this study was to prepare a high-resolution G-banded idiogram of the horse. This was necessary so that segments of individual chromosomes could be ascribed with numerical identity. The idiogram would also upgrade the present horse chromosome nomenclature. The third objective was to compare high-resolution banded chromosomes of control (nonaffected) with HME affected horses to determine whether a persistent chromosome aberration could be associated with HME.
Cytogenetics of the Domestic Horse (Equus caballus).

Diploid chromosome numbers of the domestic horse before 1959 were reported to vary greatly, from 20 to 60. Rothfels, et al. (1959) was the first to correctly identify the diploid chromosome number of the domestic horse as 64. With the advent of banding techniques, cytogenetic studies of the domestic horse have greatly increased available information on horse chromosome morphology (Buckland et al., 1976; Thacher, 1978; Ford et al., 1980; Kopp et al., 1981) and evolutionary patterns (Eldridge and Blazak, 1976; Ryder et al., 1978).

Until 1976, banded domestic horse karyotypes were published without using a standard G-banded karyotype. Therefore, placement of chromosomes was subjective resulting in several different chromosome numbering systems (Buckland et al., 1976; Eldridge and Blazak, 1976; Ryder et al., 1978; Thacher, 1978; deGiovanni et al., 1979). In 1976 an International Conference was organized to prepare and present standard G-band karyotypes of several domestic animals (Ford, et al., 1980). The Reading Conference standard domestic horse G-band karyotype was prepared from a mid-metaphase chromosome spread and the chromosomes were arranged into five groups: submetacentric (4
pairs), metacentric (8 pairs), subtelocentric (1 pair),
teleocentric (18 pairs) and the sex chromosomes. Divisions
of chromosome arms for the purpose of description were
subjective. Descriptions of bands included: staining or
non-staining bands, narrow or broad bands, and prominent,
dark or light bands.

C-bands of the domestic horse (or areas on chromo-
somes with inert genetic material) were described by
Buckland et al. (1976), and Ryder et al. (1978). Both
researchers reported centromeric heterochromatin on most
chromosomes. Exceptions to this are chromosome pair 11
with no C-bands and, chromosome pair 8 with very light C-
bands. The X chromosome has an interstitial C band on
the q arm and the Y is almost entirely heterochromatinc.
Both Buckland et al. (1976) and Ryder et al. (1978) also
reported C-band heteromorphism of chromosome pair 13 in
many horses studied. Buckland et al. (1976) reported an
interstitial band on the q arm of chromosome pair 12 and
a small C-band on the telomeric end of the p arm of
chromosome pair 1.

Kopp et al. (1981) reported Nucleolar Organizer
Regions (NORs) (areas on certain chromosomes which con-
tain genes that code for rRNA) to be located on horse
chromosomes 1, 25 and 30, with 6% of the metaphases
studied showing associations.
Thacher (1978) prepared the first domestic horse G-band idiogram using a late metaphase spread. deGiovanni et al. (1978) made chromosome measurements of 20 domestic horse metaphases and prepared an unbanded idiogram based on linear measurements and morphology of the chromosomes.

Hereditary Multiple Exostoses

Hereditary Multiple Exostosis (HME) is a bone disorder which affects the long bones and some flat bones during skeletal development, although none have been discovered on the skull. Clinically, HME has been observed in humans, horses, dogs, cats, lions and lizards. It is usually discovered early in life when bones are most actively growing. The tumors stop enlarging as bone growth slows later in life at maturity and, after that time, no new lesions develop. Except for about a 1-20% malignancy rate in humans, no malignancies have been reported in any other species.

Hereditary Multiple Exostosis is known by many different names: "Hereditary Deforming Chondrodysplasia" (Ehrenfried, 1917); "Diaphyseal Aclasis" (Keith, 1920), a name still used by the British; "Hereditary Multiple Exostoses" (Jaffe, 1943), the term most widely used; "Cartilaginous Exostoses" and "Hereditary Osteochondromatosis".
From the earliest written reports, HME was thought to be hereditary, although the pattern of inheritance has been widely disputed. Jaffe (1958) reported that one-half of the offspring of an affected person manifested HME, but that the ratio of males to females affected is about 7 to 3. Aegerter and Kirkpatrick, Jr. (1968) reported a hereditary inheritance pattern of almost 75% of the cases studied and that males are affected three times as often as females. They claimed that the father usually passed the trait to the children but there were several cases in which only the mother was afflicted. Greenfield (1975) reported a mendelian dominant form of transmission with males twice as often afflicted with HME as females. Genetic transmission of exostosis was studied in 281 Lusitanos horses by 23 stallions and 107 Arabian horses by 11 stallions by Monteiro and Barata (1980). Through the use of population genetics it was concluded that three pairs of genes are involved, one autosomal dominant, one autosomal recessive and a recessive on the X-chromosome. Gardner et al. (1975) and Shupe et al. (1979, 1981), in a study of both horses and man afflicted with HME, reported that through the use of pedigrees, the inheritance pattern was determined to be that of a single, dominant autosomal gene with 100% penetrance. It was also reported by Gardner et al. (1975) and Shupe et al. (1979, 1981) that the tumors of
horses and man are clinically and histologically similar and therefore the horse may be used as a model to study HME in human populations. Cytogenetic studies showed that horses and humans afflicted with HME have a higher occurrence of aneuploidy in cultured cells, although no persistent chromosome abnormalities were found (Shupe et al., 1979).
MATERIALS AND METHODS

Ten ml of blood was aseptically taken from each of two control (both male) and four exostosis horses (one male and three females).

Leukocytes were cultured by two methods. The first method utilized a slightly modified technique of Lin et al (1976). Five ml Hanks Balanced Salt Solution (HBSS—without Mg++ or Ca++) was added to 5 ml whole blood in a 15 ml polystyrene centrifuge tube and gently mixed to obtain a homogeneous solution. Three ml Ficoll-Hypaque was added to 2 centrifuge tubes. Five ml of the blood-HBSS mixture was carefully layered over the Ficoll-Hypaque. The centrifuge tubes were then centrifuged at 2000 rpm for 40 min, after which the top layer of the HBSS was aspirated and discarded. The leukocyte layer, the opaque band between the HBSS and erythrocytes, was then aspirated into a clean, sterile centrifuge tube. The leukocytes were washed once with 12 ml of HBSS and then recentrifuged at 800 rpm for 20 min. The cell pellet was gently resuspended in culture medium consisting of 7 ml RPMI 1640 supplemented with 10% fetal calf serum, 2% L-glutamine, 1% Pen-Strep and 1.5% PHA and cultured for 72 hrs at 37°C.

The second method was a modification of the standard 72 hr whole blood culture technique of Moorhead et al.
(1960). One-half ml of whole blood was cultured for 72 hrs at 37°C in 7 ml supplemented RPMI 1640 (10% FCS, 2% L-glutamine, 1% Pen-Strep) and 1.5% Pokeweed mitogen.

Regardless of the type of culture method used, at 72 hrs the cells were blocked at the G1-S border with 10^{-9} M methotrexate (amethopterin) for 17 - 21 hrs. At this time, the cells were centrifuged, resuspended and washed once with HBSS, recentrifuged and released from the block by resuspending in supplemented culture medium plus 10^{-9} M thymidine for 4.5 hrs. Colcemid (0.05 mcg/ml) was added for 7 min. The cells were harvested in the usual manner. This technique is a modification of a method described for human cytogenetic work by Yunis et al. (1978).

G-bands were obtained using 48 hr heat-pretreated slide preparations and placing them in a trypsin solution (.25% trypsin-0.1% EDTA-HBSS) for approximately 2 min. The slides were then rinsed in HBSS, 70% ethanol, 90% ethanol and air-dried. The slide preparations were stained with 2% Giemsa-P0_{4} buffer (pH 7.2) (Wang and Federoff, 1972).

C-bands were obtained using room-temperature aged (7 days) slide preparations by pre-treating them in 0.2M HCl for 20 min. The slides were then placed in a 37°C saturated barium hydroxide solution for approximately 4 min. The slides were then rinsed with 0.2M HCl and 2XSSC (standard sodium citrate) and placed in a moist chamber
at 37°C overnight. The slides were stained with 2% Giemsa-PO₄ buffer (pH 7.2) (Sumner, 1972).

NORs were obtained by flooding room-temperature aged slide preparations with a 1% silver nitrate solution and placing the slides in a 37°C moist chamber overnight. NORs became visible in 4-5 hrs after one drop of 1% formalin was added to the silver nitrate solution. The slides were counterstained with 2% Giemsa-PO₄ buffer (pH 7.2) (Bloom and Goodpasture, 1976).

Suitable chromosome spreads were photographed using high-contrast Kodak film Pan 2415 on a Zeiss photoscope at 1000X. The exposure time was 3-4 sec. The film was developed using Kodak products (D-19 developer and fixer) according to the manufacturer's specifications for high-contrast film. The negatives were enlarged 8.5X and prints made using Kodak Dektol developer and fixer with a water-0.05% glacial acetic acid stop bath solution used between the developer and fixer steps. The prints were then washed for 0.5 hr and placed in a print flattening solution (Pakasol) for 10 min, after which the prints were dried.

Karyotypes were prepared of G-banded, C-banded and NOR spreads of all horses studied.

For comparative purposes, one karyotype was prepared using one homologue of the control horse (male) and one
from an exostosis male.

A G-band high-resolution idiogram was prepared using three G-banded karyotypes from a control male horse.
RESULTS

Figure 1 (page 20) shows the technique used for obtaining high resolution elongated horse chromosomes. The standard mitogen, phytohemagglutinin (PHA) could not be used in horse whole blood cultures. Regardless of concentration used, PHA appeared to enhance the rouleaux effect of horse erythrocytes. The use of Lin's et al. (1976) density gradient technique for isolating leukocytes removed the red blood cells, which then allowed the use of PHA in the culture. This method increased the yield of mitoses per slide as compared to culturing whole blood stimulated with Pokeweed mitogen. Yunis' et al. (1978) technique, described for human cells, was modified for use in horses by decreasing the concentration of methotrexate and thymidine from $10^{-7}$ M to $10^{-9}$ M. A concentration of $10^{-7}$ M methotrexate resulted in extremely few mitoses and $10^{-7}$ M thymidine blocked the leukocytes instead of releasing them from the $G_1$-S border. Shortening the time of cell release and time in colcemid was necessary to obtain less contracted chromosomes. This modified technique greatly increased the number of cells in prophase, prometaphase and early metaphase.

Figure 2 (page 21) shows a diagrammatic representation of horse chromosome 1 in different degrees of contraction. The mid-metaphase chromosome is at the stage
of contraction that has been reported in most publica-
tions.

Figure 3 (page 22) presents the proposed standard
domestic horse G-band karyotype, prepared by using pro-
metaphase chromosomes some from three different karyo-
types. Autosomes are arranged into two major groups: bi-armed which are metacentric (13 pairs) and submeta-
centric and single armed acrocentrics (18 pairs).

A high resolution G-band idiogram (Figure 4, page 23) was prepared using prometaphase chromosomes from three different karyotypes. Large sections of chromosome arms were subdivided at the edges of major bands, rather than at the center of major bands.

Description of G-banded prometaphase horse chromo-
somes:

Chromosome

1. p-arm: Regions 1 and 2 each contain four positive bands. There are two characteristic positive bands in region 1 (14 and 18) and two characteristic negative bands in regions 2 (23 and 27).
q-arm: Region 1 contains five positive bands with two characteristic positive bands (18 and 110). Region 2 has six positive bands with two characteristic positive bands (23 and 25).

2. p-arm: Region 1 contains four positive bands, all approximately evenly spaced.
q-arm: Region 1 has three positive bands with a wide negative band (13). Region 2 has three positive bands with a characteristic positive band (24).

3. p-arm: Region 1 has three positive bands with a
wide positive central band (14).
q-arm: Region 1 has four positive bands with two characteristic positive bands (14 and 18). Region 2 has four positive bands with a characteristic negative band (21).

4. p-arm: Region 1 has two positive bands of which 12 is characteristic.
q-arm: Region 1 contains four positive bands. The characteristic bands are 12 and 14, and 16 and 18, with each pair fusing into one band with more contracted chromosomes. Region 2 has four positive bands. There are two characteristic negative bands (21 and 25).

5. p-arm: Region 1 has four positive bands with a characteristic negative band (15).
q-arm: Region 1 contains three positive bands with 16 being characteristic. Region 2 has two positive bands with a wide negative band at 21.

6. p-arm: Region 1 has four positive bands with a characteristic negative band (15).
q-arm: Region 1 has three positive bands with 14 and 16 fusing into one wide positive bands of which two are characteristic (22 and 26).

7. p-arm: Region 1 has three positive bands with one central characteristic positive band (14).
q-arm: Region 1 has three positive bands with bands 14 and 16 fusing into one band as the chromosome contracts. Region 2 has three positive bands with a characteristic wide negative band (24).

8. p-arm: Region 1 has 3 positive bands all of which are approximately evenly spaced.
q-arm: Region 1 has three positive bands with a wide negative band (22).

9. p-arm: Region 1 has three positive bands of which 12 is characteristic.
q-arm: Region 1 has two positive bands both of which fuse into one band as the chromosome contracts. Region 2 has three positive bands with a characteristic negative band (24).

10. p-arm: Region 1 has three positive bands with 16 as the characteristic feature.
q-arm: Region 1 has two positive bands, both evenly spaced. Region 2 has three positive bands
with a characteristic negative band (22).

11. p-arm: Region 1 has one positive band distally located (12).
q-arm: Region 1 has four positive bands with 22 being characteristically darkly staining.

12. p-arm: Region 1 has one positive band (12).
q-arm: Region 1 contains two positive bands, both of which fuse into two bands as the chromosome contracts.

13. p-arm: Region 1 has two positive bands of which 12 is characteristic.
q-arm: Region 1 has three positive bands of which 12 and 14 are characteristic.

14. q-arm: Region 1 has three positive bands with 14 and 16 fusing into one band as the chromosome contracts. Region 2 has five positive bands with a characteristic wide negative band (24).

15. q-arm: Region 1 has four positive bands. The characteristic bands are 12 and 14, and 16 and 18, with each pair fusing into one band as the chromosome contracts. Region 2 has four positive bands with 21 and 23 fusing into one band as the chromosome contracts.

16. q-arm: Region 1 has four positive bands. Bands 16 and 18 are usually fused in most preparations. Region 2 has four positive bands with 23 and 25, usually fused, as characteristic features.

17. q-arm: Region 1 has three positive bands with bands 12 and 16 being characteristic. Region 2 has five positive bands. Band pairs 21 and 23, and 27 and 29 each fuse into one characteristic band as the chromosome contracts.

18. q-arm: Region 1 has three positive bands with a characteristic negative band (17). Region 2 has five positive bands. Band pairs 21 and 23, and bands 27 and 29 fuse into one characteristic band as the chromosome contracts.

19. q-arm: Region 1 has five positive bands with two characteristic positive bands (18 and 110). Region 2 has three positive bands with a central characteristic negative band (23).
20. q-arm: Region 1 has three positive bands of which bands 14 and 16 are characteristic. Region 2 has three positive bands and two characteristic negative bands (21 and 23).

21. q-arm: Region 1 has two positive bands both evenly spaced. Region 2 has four positive bands of which 25 is characteristic.

22. q-arm: Region 1 has two positive bands of which 14 is characteristic. Region 2 has three positive bands of which 23 and 25 are characteristic.

23. q-arm: Region 1 has four positive bands of which 14 and 16 fuse into one band as the chromosome contracts.

24. q-arm: Region 1 has four positive bands with a characteristic negative band (15).

25. q-arm: Region 1 has three positive bands all approximately evenly spaced and band 14 slightly wider than the other two.

26. q-arm: Region 1 has five positive bands of which 14, 16 and 18 are usually fused in most preparations.

27. q-arm: Region 1 has three positive bands with a characteristic negative band (13).

28. q-arm: Region 1 has four positive bands with a characteristic wide positive band at 12.

29. q-arm: Region 1 has four positive bands of which 14 and 16 are characteristic and fuse as the chromosome contracts.

30. q-arm: Region 1 has two positive bands with band 12 more darkly staining and wider than 14.

31. q-arm: Region 1 has three positive bands, all of which are approximately evenly spaced.

X p-arm: Region 1 has two positive bands with a characteristic negative band (15). Region 2 has three positive bands with band 21 being characteristic.

q-arm: Region 1 has three positive bands with a characteristic negative band (13). Region 2 has
four positive band and a characteristic negative band at 21.

Y q-arm: Region 1 has five positive bands with a wide characteristic band at the telomeric end (19).

A previously published study on domestic horse NORs reported them to be on chromosome pairs 1, 25 and 30 (Kopp et al., 1981). In this study many chromosome spreads had associations between homologues of chromosome pair 1 and associations between 1 and one of the smaller acrocentric chromosomes. Karyotypes prepared from spreads showing such associations revealed that chromosome 31 is involved in the association (Figures 5 and 6, pages 24 and 25). No associations were observed between chromosomes 1 or 31 to chromosome 25. All horses studied had NORs located on chromosomes 1, 15 and 31 (Figure 7, page 26). One homologue of chromosome 25 consistently showed less silver staining than the other homologue in all horses studied.

C-band karyotypes prepared from all horses studied showed that the heterochromatin was located at the centromeric areas in most of the chromosomes (Figure 8, page 27). Chromosome pair 11 had little heterochromatin and, in many C-band karyotypes, chromosome pair 12 possessed heteromorphic C-bands. The X-chromosome contained an interstitial C-band on the q-arm and the Y-chromosome was
almost totally heterochromatic with a very small amount of euchromatin at the telomeric end. This study did not show an interstitial band on chromosome 12 or a C-band on the telomeric end of the p arm of chromosome pair 1 as described by Buckland et al. (1976).

Figure 9 (page 28) shows an NOR karyotype of a mare afflicted with exostosis. NORs are located on chromosomes 1, 25 and 31. Figure 10 (page 29) shows a male exostosis NOR karyotype.

Figure 11 (page 30) shows a C-band karyotype of a mare afflicted with exostosis. Figure 12 (page 31) presents a C-band karyotype of a male affected by exostosis. Results are identical to the control horse C-bands.

Figure 13 (page 32) presents a G-band comparison between control and exostosis horse chromosomes. Homologues of the two horses appear identical.
HIGH RESOLUTION ELONGATED CHROMOSOMES

LEUKOCYTES  (density gradient - isolated white blood cells, PHA)
or
WHOLE BLOOD  (pokeweed mitogen)

\[ \downarrow \] 72 hrs

CELLS ARRESTED  \((10^{-9} \text{M amethopterin})\)

\[ \downarrow \] 17-21 hrs

CELLS RELEASED  \((10^{-9} \text{ thymidine})\)

\[ \downarrow \] 4.5 hrs

COLCEMID TREATMENT

\[ \downarrow \] 7 min

HARVEST

Figure 1. Technique used for obtaining high resolution elongated horse chromosomes.
Figure 2. Diagrammatic representation of horse chromosome 1.
Figure 3. Proposed standard domestic horse G-band karyotype.
Figure 4. High resolution G-band idiogram.
Figure 5. Metaphase spread showing 1-31 association.
Figure 6. Karyotype prepared from I-31 association.
Figure 7. Control horse NORs.
Figure 8. Control horse C-bands.
Figure 9. Exostosis mare NORs.
Figure 10. Exostosis male horse NORs.
Figure 11. Exostosis mare C-bands.
Figure 12. Exostosis male C-bands.
Figure 13. G-band comparison between control and exostosis horse chromosomes.
DISCUSSION

The cell culture method described herein is an improved leukocyte culture technique that consistently provides numerous elongated chromosomes which are necessary for studies that utilize high resolution G-banding.

In Figure 2 (page 21) chromosome stages range from late metaphase to late prophase which contains the greatest number of bands. Late prophase chromosomes, however, are the most difficult to work with because only 2% of the chromosome spreads per slide are at this stage of contraction. Also, late prophase spreads have many chromosomes that overlap which result in distorted banding patterns. Six percent of the chromosome spreads were at prometaphase, and it is this stage of contraction that the chromosome appears to have the greatest utility. Prometaphase chromosomes provide numerous recognizable spreads with few overlaps. The prometaphase chromosome was therefore used in the preparation of the proposed karyotype and idiogram.

The proposed standard G-band karyotype contains considerably more bands than previously described G-band karyotypes of the horse. The number of bands in comparison to the Reading Conference standard has been increased from 442 to 852. This 2-fold increase provides much greater resolution for linear differentiation of specific
regions within a chromosome and therefore should allow greater accuracy in comparative studies.

The arrangement of chromosomes in the proposed karyotype varies from the method proposed by the Reading Conference (Ford et al., 1980). There are a few basic problems with the Reading Conference arrangement of chromosomes. First of all, the differences between the submetacentric and subtelocentric groups are not clearly defined and therefore does not appear to be an advantage in separating these two groups. Secondly, the delineation between these two groups becomes very obscure and cumbersome when working with horse chromosomes at an earlier stage of contraction than at mid-metaphase and late metaphase. My experience indicates that the rate of contraction of the p arm varies with the degree of contraction of the chromosome and depending on the stage of contraction will be the determining factor as to the group the chromosomes will be placed into. My last concern is the grouping considered telocentric. Telocentric chromosomes are considered to be unstable and their occurrence in higher organisms are believed to be rare. This leaves some question as to whether the group of telocentric chromosomes as defined by the Reading Conference are true telocentrics. For these reasons I chose to simplify the Reading Conference classification of the horse karyotype by placing the autosomes into two
major groups: bi-armed which are metacentric and sub-metacentric and single armed acrocentrics.

The proposed high resolution G-band idiogram varies from the widely used guidelines of the Paris Conference (1975) in which nomenclature for human chromosomes was standardized. In our idiogram, large sections of chromosome arms were subdivided at the edges of major bands, rather than at the center of major bands. At different stages of contraction, this delineation is not altered by fusion of bands during chromosome contraction.

The difference between this study and Kopp's et al. (1981) study of horse NORs is the method of numbering horse chromosomes. Kopp's et al. (1981) published report presented a metaphase spread with arrows pointing to the NORs. If chromosomes are to be numbered, then perhaps karyotypes should be prepared from metaphase spreads to insure correct placement of chromosomes according to size and morphology.

Although C-bands in this study generally agreed with Buckland et al. (1976), two minor differences were found. Buckland's et al. (1976) report of a C-band on the p arm of chromosome 1 and an interstitial band on chromosome 12 appears to be due to individual variation only and does not generally apply to all horses studied.

No differences were found on NORs, C-bands and G-
bands between control and exostosis horses. Hereditary Multiple Exostosis is most likely due to a simple gene effect, which would be too miniscule to be observed by gross chromosome studies. Biochemical studies, therefore, will be necessary to locate the chromosome that carries the gene(s) causing HME.


Thacher, J.E. 1978. The normal karyotypic characteristics of the domestic horse (Equus