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THE CHROMOSOMES AND NEUTROPHIL NUCLEAR APPENDAGES OF *HIPPOPOTAMUS AMPHIBIUS* LINNAEUS 1758

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Introduction

The recent artificial reduction of the numbers of hippopotami in the Kruger National Park offered an opportunity to undertake karyological studies. Such studies have not yet been undertaken on this species. The only chromosome determinations done on members of the suborder Suiformes have been on those in the family Suidae (Makino, 1951), viz. *Pecari tajacu* (2n = 30); *Sus scrofa domesticus* (2n = 38, Gerneke, 1964) and *Sus vittatus leuconystax* (2n = 40).

Method and Material

The hippopotami were shot while in the water, and some two to three hours later they rose to the surface and were towed to the river bank. Bonemarrow was collected from the spinous process of the first or second thoracic vertebra. It was treated as described by Gerneke (1964). A hand-operated centrifuge was used while water heated to 37° C served as an incubator.

Preparations were made from four bulls and two cows. The interval between death and the collection of marrow varied from one to three-and-a-half hours. The only preparations that were successful were those from one male (interval one hour) and from one female (interval one-and-a-quarter hours). The female was shot on the sand, but due to difficulty in traversing the rugged terrain, collection of marrow could not be performed sooner. Out of the thirty preparations prepared from her, only one yielded two suitable spreads. Those of the male were more successful and yielded 28 spreads.

Results

The chromosome number in all 30 spreads was constantly found to be 2n = 36.

Karyogram analysis (Fig. 1) based on the Denver system (Böök *et al.*, 1960) revealed the following grouping and morphology:—

Group 1–4

The chromosomes were all large. The first three pairs had median centromeres whereas the last one was acrocentric.

Group 5–8

They were smaller than the previous, 5 being metacentric, 6 slightly submedian and 7 and 8 acrocentric.

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Group 9–12

Chromosomes 9 and 10 had median centromeres whereas 11 and 12 were submedian.

Group 13–17

This group comprised five pairs of fairly short metacentric chromosomes.

The x-chromosome was submedian. It fitted between chromosomes 8 and 9. The y-chromosome was the smallest member and thus very easily distinguished; it was also submedian.

TABLE 1.—Results of nuclear sexing in blood smears of male and female hippopotami.

Sex	Drumsticks	Vesicular Appendages	Small Clubs	Sessile Nodules	No. of neutrophils counted
♀	128	3	0	125	2,000
♂	0	50	19	26	2,000

Nuclear sexing was exceptionally clearcut in blood smears (Table 1). Drumsticks were numerous (64/1000) in neutrophils of females and very clearly defined. In the male no typical drumsticks were encountered. An occasional structure resembling a drumstick was found, but in every case it contained a central vacuole and was, therefore, considered to be an atypical " racket " structure. Vesicular appendages (" racket " structure minus a chromatin strand attachment) were fairly numerous (25/1000) in male neutrophils (Fig. 2 to 5). They varied in size, were always attached to the nucleus and surrounded by a thin membrane with the same staining reactions as nuclear chromatin. They contained a homogeneous substance, most probably nuclear sap, giving a light blue staining reaction with Ramanovsky-type dyes. From the various morphological shapes of the vesicular appendage a progressive sequence in its formation may be postulated: a small club gives rise to a typical " racket " structure (Fig. 2). Then its " handle " is either split in two and incorporated as two granules or remains single and is incorporated as a single granule. Its point of attachment to the nucleus broadens and thus the appearances in Fig. 3 to 5 are formed. These latter forms of the appendage were most numerous, that presented in Fig. 2 was rather rare. The " handle " may remain attached to the nucleus in which case the vesicular appendage is agranular. Such agranular vesicular appendages did occasionally occur in males and even have been noted very rarely in blood smears of female hippopotami. In the latter case they were always without granules.

DISCUSSION

A constant difficulty in undertaking chromosome determinations from bone-marrow preparations is the usual lack of sufficient initial mitoses. It is significant that in the preparations prepared after the animal had been dead for longer than approximately one hour, no mitotic spreads nor mitoses were encountered. As all preparations were systematically scrutinised, it cannot be due to faulty technique (some preparations with identical technique were successful) but due to the fact that mitoses stop being initiated very soon after death. This phenomenon most

likely may be ascribed to the absence of oxygen. Some experimental evidence listed by Hughes (1951) suggests oxygen as one of the necessary substances for the initiation of mitosis. As the amount of oxygen in the blood diminishes, mitosis would be initiated in fewer cells whereby the possibility of finding suitable spreads in bonemarrow preparations made at intervals after death would be progressively diminished. The cells in which mitosis is initiated do not need oxygen to complete division (Ham & Leeson, 1961). The time given for prophase (± 70 min) and metaphase (± 20 min) to be completed approximates 1·5 hours [based on the assumption that the times for bonemarrow cells correspond approximately to those worked out for intestinal epithelium (Ham & Leeson, 1961)]; it fits in very well with the observation that preparations made 1·25 hours after death present only two spreads per 30 preparations. The bonemarrow cells, although still alive at the time of collection, are apparently inhibited from undergoing mitosis. It appears logical to conclude that for suitable metaphase spreads bonemarrow must be collected at most within one hour after death.

The vesicular appendages noted in the male hippopotami (Fig. 2 to 5) have to my knowledge never been described. In interpreting such nuclear phenomena, due consideration must be given to conclusions made in recent studies by Marcel Bessis (1964) and others on changes occurring during the period of death agony and death itself. These vesicular appendages very much resemble his " paranuclear vacuoles " studied under phase contrast cinematography and considered indicative of cell agony and death. However, he described no accompanying granules. Unfortunately no blood smears made from living animals were available for further investigation. Nevertheless it does appear as if the vesicular appendages are present prior to cell death.

Conclusions

The chromosome number of the hippopotamus is $2n = 36$, occurring in five morphological groups according to the Denver system. The x and y chromosomes are distinct. The appendages in male and female hippopotami are so characteristic of each sex that they form an even more dependable criterion for sexing than in humans.

Summary

The number of chromosomes of the hippopotamus is $2n = 36$. Their morphology and grouping are shown in a karyogram. Sex determination is based on the x-y mechanism. Nuclear sexing on the appearance of neutrophil nuclear appendages is clearly defined owing to the presence of numerous drumsticks in females and their absence in males. The differentiation is further enhanced by the presence of peculiar vesicular appendages especially in neutrophils of males, although the possibility that their formation may be due to changes occurring during death, is not excluded.

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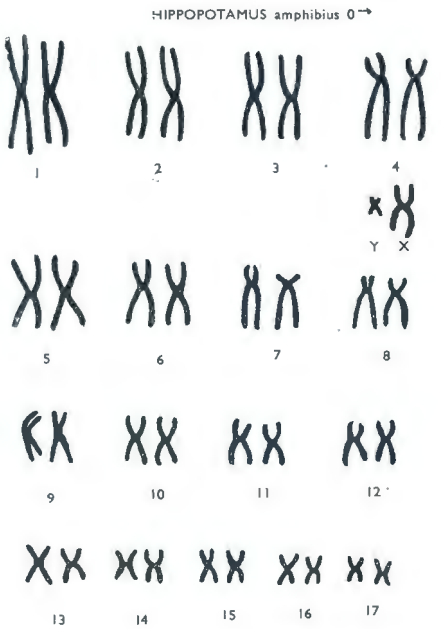

Fig. 1

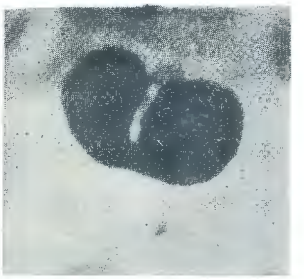

Fig. 2

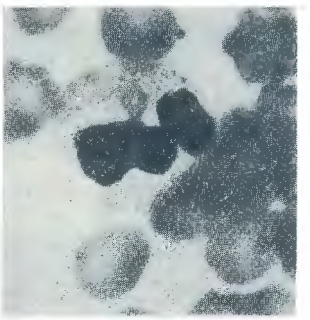

Fig. 3

Fig. 4

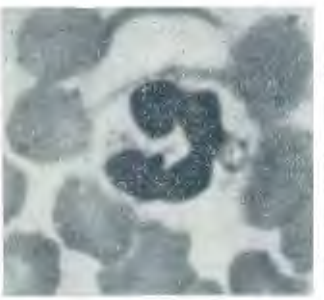

Fig. 5

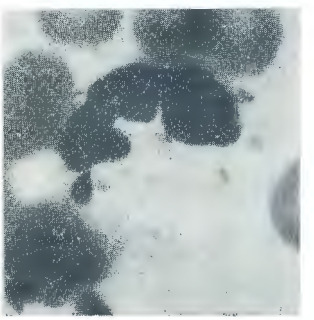

Fig. 6

FIG. 1.—A karyogram compiled from a camera lucida drawing of a metaphase spread from the bonemarrow of a male hippopotamus

FIG. 2–5.—Vesicular nuclear appendages in neutrophils of male hippos. Fig. 2 presents a typical "racket"; Fig. 3 an expanded racket with a slight proximal granule; Fig. 4 and 5 excessively expanded rackets with one and two granules respectively

FIG. 6.—A typical darkstaining drumstick in a neutrophil from a female hippopotamus is shown. A vesicular appendage is also present but without any granules. Such appendages are very rare in females