Cytogenetic Studies of Dolphin (*Tursiops truncatus*) by an Extended Tissue Culture Technique

Dolphin (*Tursiops truncatus*) is an aquatic mammal of the family Delphinidae. Cytogenetic studies have been reported on this species[1,2]. As recorded in the literature, the preparation of chromosomes, as well as percent mitosis of leukocytes from peripheral blood of this species, was not entirely satisfactory. We present results utilizing a slight modification of the usual tissue culture technique which surmounts some of these difficulties.

Materials and methods. A male and a female member of *T. truncatus* were used in this study. These two sea mammals are captive specimens of an oceanarium collection (Sea-Arama of Texas, Galveston, Texas). They were captured in the Gulf of Mexico within 12 miles of the Texas coast. Peripheral blood from each dolphin was drawn by venipuncture into a sterile heparinized (20 U/ml of blood) syringe. Leukocytes were separated by gravity centrifugation. Differential cell counts were made, and 1×10^6 leukocytes were suspended in 2.5 ml of culture medium (Eagle's MEM with L-glutamine and 20% heat inactivated fetal calf serum, Erythromycin 50 µg/ml, Streptomycin 100 µg/ml, pH 7.2 to 7.5 adjusted with $NaHCO_3$. (All reagents were obtained from Grand Island Biological Laboratories, Grand Island, N.Y.). Phytohaemagglutinin-M (Difco Laboratories) was reconstituted with phosphate buffer saline and was added to the medium (0.1 ml/tube). Triplicate culture tubes with loosely fitted stainless steel caps were incubated at 37 °C in a water saturated atmosphere of 95% air, 5% CO_2. The incubation period varied from 1 day (24 h) to 7 days. During these cultivation periods additional nutrients were not added nor was the media changed. 2 h before harvesting, colchicine (Lilly Co.) was added to make a final concentration of 1 µg/ml of media. The cells were harvested daily from 1 to 7 days, pretreated (1% sodium citrate for 20 min), fixed (acetic-alcohol, 1:3 for 30 min), spread by flame dry technique, and stained with 0.05% Giemsa. Karyotypes were determined from 6th and 7th day cultures.

Results. Well-defined metaphase plate counts from the cultures of different incubation periods are presented in the Table. A modal diploid chromosome number of *T. truncatus* was found to be 44 which is in close agreement with the findings of other investigators[1,2]. Chromosomes are sub-classified into 4 groups and one pair of sex chromosomes as shown in Figures 1 and 2. Group A consists of 5 large chromosome pairs which are sub-metacentric; group B has 6 smaller sub-metacentric pairs; group C has 5 metacentric pairs; and group D has 5 pairs of acrocentric chromosomes. The last pair of group D chromosomes is smallest among the autosomes. Sex chromosomes are *X*, *Y* type. The *X* is submetacentric and is similar in size to the first pair of group B (Figures 1 and 2). The *Y* is a minute element that in favorable preparations can be identified as acrocentric. With microscopy, this acrocentric structure is quite distinct; unfortunately photographic results do not clearly define their aspect.

Discussion. As noted in the experimental results (Figure 1), it appeared in this study that the *Y* chromo-

Response of dolphin blood leukocytes to various incubation periods

Days of incubation	Percent mitosis[a]	Chromosomes per cell	Metaphase spread
1	–	–	–
2	–	–	–
3	–	–	–
4	–	–	–
5	17.3	44	Good
6	20.8	44	Very good
7	21.2	44	Very good

[a] Percent mitotic value for each incubation period is based on a total of 2000 cells count.

1 D. A. Duffield, S. H. Ridgway and R. S. Sparkes, Nature *213*, 189 (1967).
2 K. H. Walen and S. H. Madin, Am. Naturalist *99*, 349 (1965).

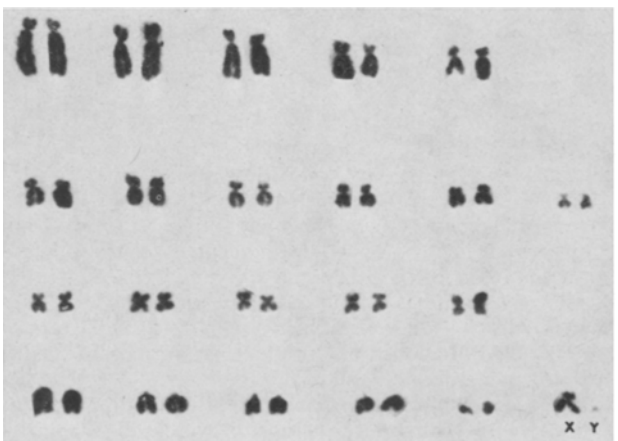

Fig. 1. Male karyotype of *Tursiops truncatus*.

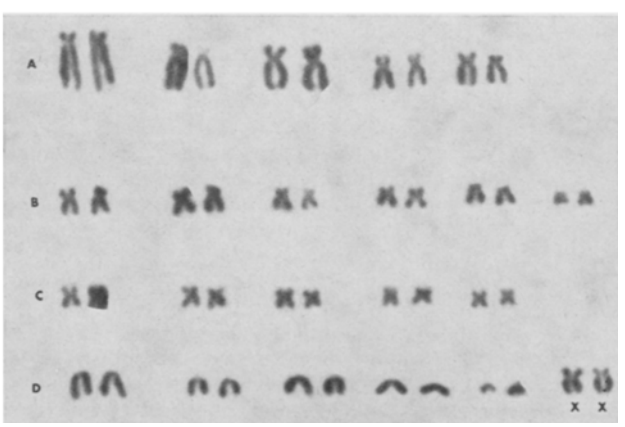

Fig. 2. Female karyotype of *Tursiops truncatus*.

some of the male is acrocentric rather than submetacentric[1]. In contrast to the karyotype described by DUFFIELD et al.[1], we found a difference within the autosome groups. Group B has 6 pairs of smaller submetacentric chromosomes rather than 7, and group C has 5 pairs of metacentric chromosomes rather than 4 (Figures 1 and 2).

For some unknown reason 6th and 7th day in vitro cultures of leukocytes from *T. truncatus* gave a very good mitotic response and metaphase chromosome spread. Other investigators[1] have attempted to change the ingredients of the tissue culture media. These alterations resulted in a reduction of mitotic response. From these limited studies it seems that the peripheral blood leukocytes from *T. truncatus* or aquatic mammals may require longer incubation periods or more specialized treatment than leukocytes from other mammals in order to obtain good mitotic response and satisfactory chromosomal preparations. Further experiments are under way with other aquatic mammals which might explain the necessity of longer incubation periods[3,4].

Zusammenfassung. Modifizierte Gewebekulturtechnik für Chromosomenuntersuchungen aus peripherem Blut beim Delphin *Tursiops truncatus*.

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