Aquatic Mammals 1991, **17.2,** 71–75

Serum alkaline phosphatase—changes in relation to state of health and age of dolphins

M. B. Fothergill, C. A. Schwegman, P. A. Garratt, A. Govender and W. D. Robertson

South African Association for Marine Biological Research, Sea World, Durban, P.O. Box 10712, Marine Parade, 4056, South Africa

Summary

It is proposed that serum alkaline phosphatase (A.P.) levels may be used as an indication of a dolphin's state of health. Base values for individuals are taken to be those at which animals appear to be healthy, i.e. appetite, behaviour, and other routinely monitored signs are normal. Marked decreases in serum A.P. levels, occurring within a short time span, are usually indicative of disease. Serum A.P. levels in dolphins may follow a pattern relative to age, as is found in man, and may also provide a rough guide to assessing state of maturity.

Introduction

The alkaline phosphatases (A.P.) are a group of enzymes which hydrolyse phosphates at an alkaline pH. In man the activity of A.P. measured, which includes that of isoenzymes, is found in the bone, liver, kidney, intestinal wall, lactating mammary gland and placenta (Zilva & Pannall, 1984). In cetacea the tissue activity of A.P. has so far been examined only in the Harbour porpoise, *Phocoena phocoena* (Geraci & St Aubin, 1979). It is concentrated in the kidney and adrenals with slight activity in the liver. Alkaline phosphatase variability in man and dolphins appears to be lower than in sheep and cattle (Cornelius, cited by Andersen, 1968) and has been suggested by Andersen (1968) to be of diagnostic value. The possibility of using serum A.P. levels as an early warning of pending disease was suggested by Hammond (1980) for the bottlenose dolphin, *Tursiops truncatus*. He reported that values exceeding 1000 units were commonly found in healthy animals while values below 300 units were usually associated with disease. The relationship between A.P. levels and animal health was investigated in dolphins at Sea World, Durban.

In man serum A.P. levels vary with age (Berkow, 1982). An appreciable difference in the activity of this enzyme has also been found in the serum of young and adult *Phocoena phocoena* (Andersen, 1968). Andersen (op. cit.) therefore proposed that A.P. levels may be used as an indicator of physical maturity in *Phocoena phocoena* and other mammals which have similar A.P. activity. The relationship between age and A.P. levels in dolphins was also investigated in this study.

Methods

Since 1976 blood samples have been collected from dolphins resident at Sea World. Species maintained at this institute include Indian Ocean bottlenose dolphins, *Tursiops aduncas*, Atlantic Ocean bottlenose dolphins, *Tursiops truncatus*, and Cape Dusky dolphins, *Lagenorhynchus obscurus*. From 1976 to 1979 most samples were collected when a dolphin was suspected of being ill. From 1979 to 1990 samples were collected on a monthly basis as well as during periods of illness. Samples were obtained by venipuncture from the tail fluke using an 0.80 × 30 mm Venoject multisample needle (Terumo) and collected into VAC-U-TEST tubes (Radem Laboratory Equipment) with no anticoagulant. Blood was allowed to clot at room temperature before being centrifuged to obtain serum samples. A.P. levels were determined in an external laboratory by two different analytical techniques. The first method was described by Technicon Instruments Corporation (1971). This method is an automated p-nitrophenyl phosphate alkaline phosphatase procedure for the Auto Analyzer. The normal range obtained for man using this method is 30–85 U/l. We refer to this method as the Autotest. The second method, referred to as the Monotest, is an 'optimized standard method' conforming to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie. This is a colorimetric method marketed by Boehringer Mannheim (1979) using p-nitrophenyl phosphate buffer. The normal range for man is 73–207 U/l.

Results

Health related serum A.P. changes

Observations on the three species of dolphin were made over 12 years. It was found that during periods

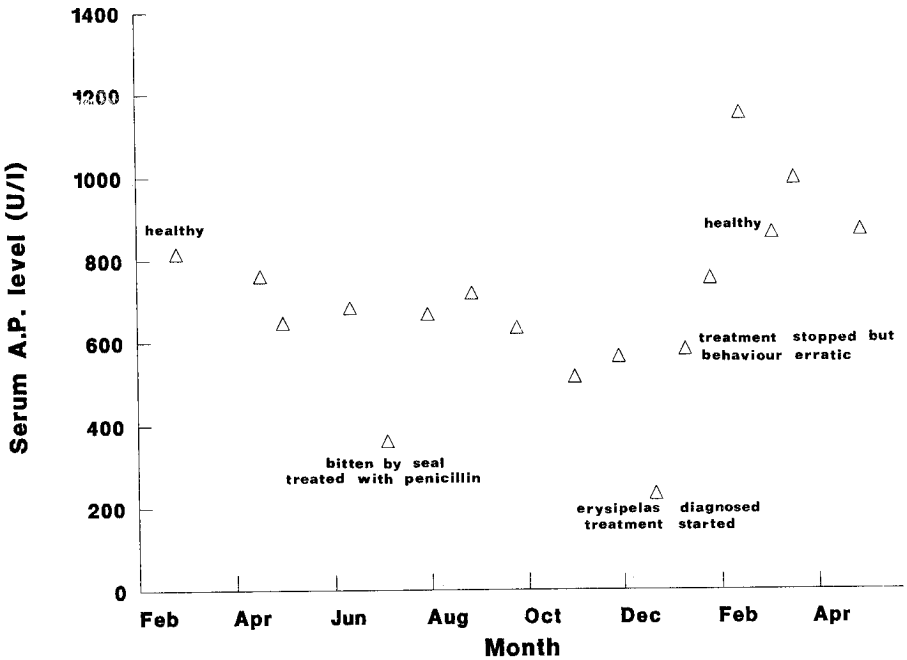

Figure 1. An adult female *Lagenorhynchus obscurus*, showing low A.P. levels after a seal bite and during an Erysipelas infection.

of illness when haematological parameters indicated viral or bacterial infection, the corresponding serum A.P. levels were generally low compared with normal base levels. There were a few exceptions when serum A.P. levels were greater than normal. The amount by which serum A.P. levels declined appeared to correspond with the severity of infection. The following are representative examples.

An adult female *Lagenorhynchus obscurus* showed a marked drop in A.P. levels on two occasions (Fig. 1). The first coincided with an infection caused by a bite from a Cape Fur seal and the second coincided with a positive diagnosis of Erysipelas. On both occasions she was successfully treated with penicillin. There was a return to normal A.P. levels after treatment.

A second adult female *Lagenorhynchus obscurus*, when considered healthy, had serum A.P. levels of 900–1000 U/l (Autotest) (Fig. 2). She manifested erratic behaviour and a reduced appetite at serum values of 600–650 U/l and at a value of 186 U/l the animal had stopped eating. One day prior to death the serum A.P. was 34 U/l. Autopsy revealed a Klebsiella pneumonia with septicaemia.

Age related A.P. changes

Blood samples taken during periods of good health from three young dolphins born in captivity (Fig. 3) indicate that serum A.P. levels of young bottlenose dolphins are many times greater than those of adults (Fig. 4). Serum A.P. levels decline steadily during the first few years (Fig. 3) but in adulthood they appear to level off (Fig. 4).

Discussion

Our observations indicate a relationship between disease and serum A.P. levels in dolphins. On many occasions lower serum A.P. levels have served as an early warning of impending illness. We have also found this relationship useful in determining whether abnormal behaviour, often exhibited by dolphins, is due to illness or social factors. If serum A.P. levels are found to be normal, we tend to attribute the abnormal behaviour to social factors. If serum A.P. levels have dropped over a short period of time, even though other diagnostic parameters have remained normal, we consider the dolphin ill but as yet undiagnosed. A.P. levels can thus assist in decisions on handling and training.

The exceptions, where levels were greater than base line levels during illness, were considered to be the result of drug therapy and in two cases liver involvement was suspected. It is suggested that results should be interpreted in the light of clinical and therapeutic drug history as many conditions, drugs and chemicals affect A.P. determinations in man (Berkow, 1982). It is also stressed that, as there is

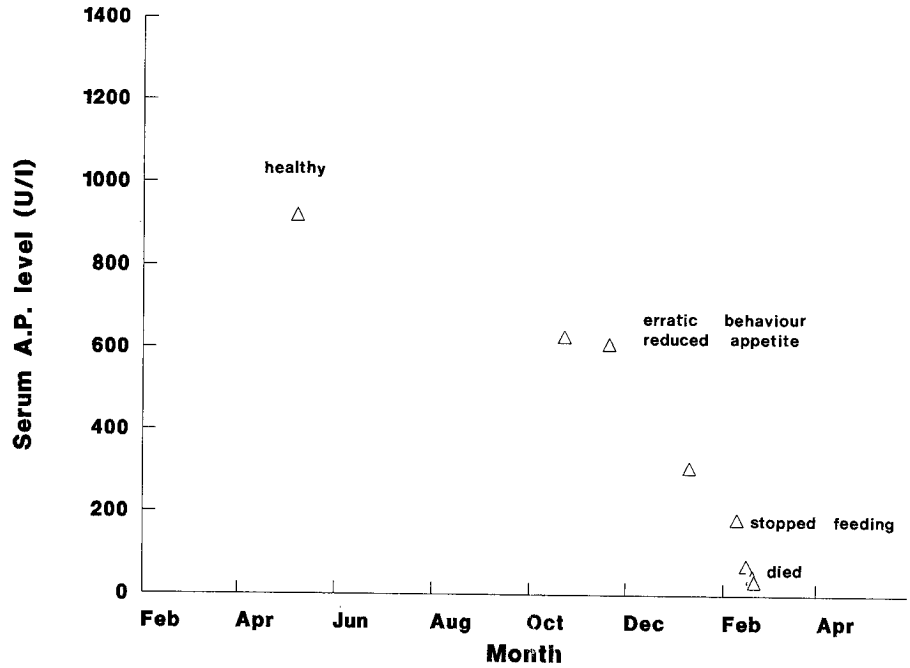

Figure 2. Serum A.P. levels of an adult female *Lagenorhynchus obscurus* which died of Klebsiella pneumonia with septicaemia.

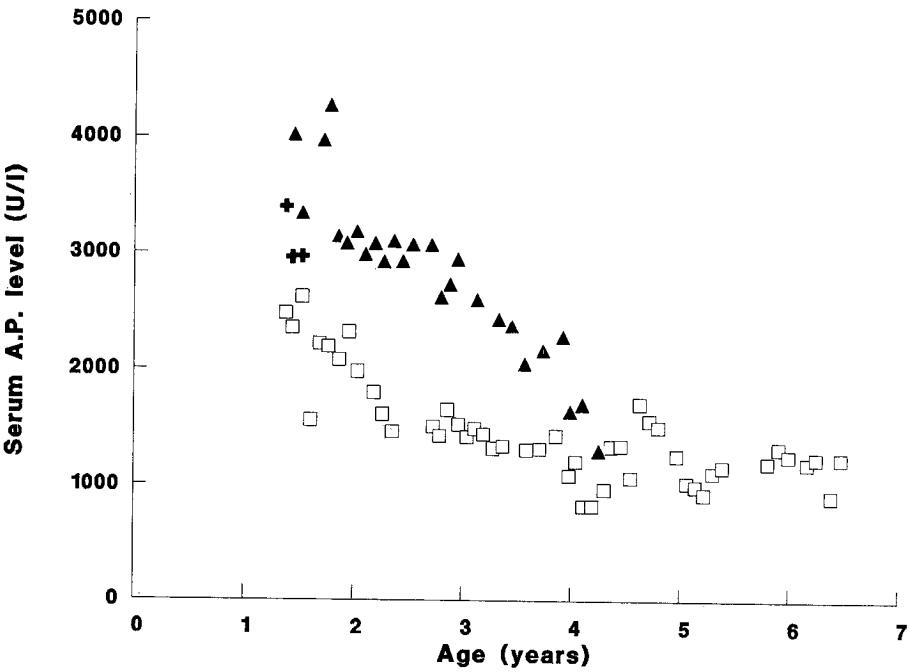

Figure 3. Serum A.P. levels taken during periods of good health from three young bottlenose dolphins born at Sea World Durban. + Kani, a male *Tursiops truncatus* showing high levels at an early age; □ Kelpie, a male *Tursiops truncatus* and ▲ Freya, a female *Tursiops truncatus/aduncas* both showing high values declining with age.

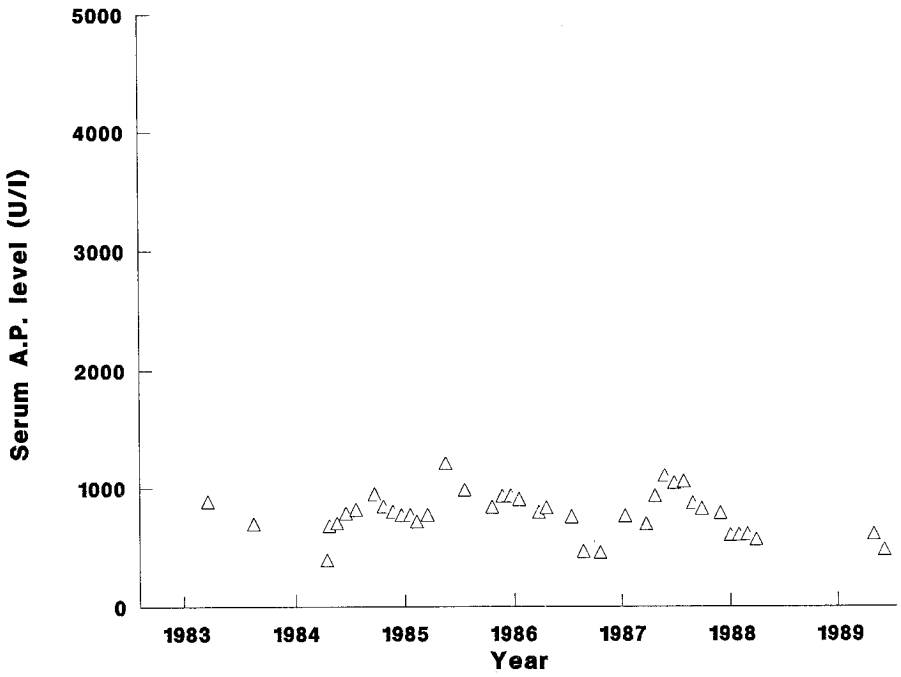

Figure 4. Serum A.P. levels taken during periods of good health from an adult female *Tursiops truncatus*.

great variation in analytical techniques, comparisons between values from an individual dolphin, or between different members of a group, can only be made if all analyses are carried out by the same laboratory.

The value of serum A.P. levels in assessing the maturity of dolphins is not yet clear but our data confirm that A.P. levels in younger animals are many times greater than those of the adults (Anderson, 1968). Limited data indicate that these levels decrease steadily between 17 months and about four years after which they appear to begin levelling off (Fig. 3). Values presented in Fig. 3 were measured in the same laboratory using the same analytical technique. Despite this, levels for 'Freya' are substantially greater than those for 'Kelpie'. This could be attributed to normal individual variation or difference in gender, or the fact that 'Frey' is a hybrid. Further investigation of this aspect is warranted and is now feasible in view of the greatly improved breeding success rate in captive dolphins worldwide.

In cetacea the isoenzymes of A.P. have not yet been identified (de Monte & Pilleri, 1984). There is thus a need to analyse these isoenzymes to ascertain the mechanisms underlying the low A.P. levels observed in sick dolphins and higher levels in younger dolphins. This may further confirm the suggestion made by Andersen (1968) and Clarke (1990) that the high levels in younger dolphins can be attributed to increased activity at the level of the osteoblasts, as is the case in man (Zilva & Pannall, 1984).

Acknowledgements

We thank our colleagues in the Dolphinarium for their help with the collection of blood samples and the staff of the Department of Chemical Pathology, Addington Hospital for laboratory assistance.

References

Andersen, S. (1968) Physiological ranges of bloodchemical parameters in captive harbour porpoise, *Phocoena phocoena* (L). *Nord Veterinaermed.* **20,** 267–278.

Berkow, R. ed. (1982) The Merck Manual, 14th Ed., 820–826.

Boehringer Mannheim GmbH Diagnostica (1979) Alkaline phosphatase opt. EC 3.1.3.1. Colorimetric method. (Package insert for Monotest).

Clarke, J. P. B. (1990) Helpful hints of basic blood values in *Tursiops truncatus*. *Soundings* **15(3),** 20–22.

De Monte, T. & Pilleri, G. (1984) Cetacean hematology. IV. Substrates, enzymes, electrolytes and other organic and mineral substances present in the circulation. *Investigations on Cetacea* **16,** 82–121.

Geraci, J. R. & St Aubin, D. J. (1979) Tissue sources and diagnostic value of circulating enzymes in cetaceans. *J. Fish. Res. Bd. Can.* **36,** 158–163.

Hammond, D. D. (1980) The Orient: Information: The key to our future. In: Jenkins, R. L. & Halusky, J. G. eds. *Aquatic animal medicine: a state of the art,* Conference proceedings, Florida, 25 april 1979. Florida, Sea Grant College: 52–57.

Technicon Instruments Corporation (1971) Alkaline phosphatase. (Package insert for AutoAnalyzer AA11-06, November 1970).

Zilva, J. F. & Pannall, P. R. (1984) *Clinical chemistry in diagnosis and treatment,* 4th ed. London: Lloyd-Luke (Medical Books), 374–376.