Comp. Biochem. Physiol. Vol. 78A, No. 1, pp. 167–173, 1984
Printed in Great Britain

0300-9629/84 $3.00 + 0.00

PATHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF STRESS DURING CAPTURE AND TRANSPORT IN THE JUVENILE DUSKY SHARK, *CARCHARHINUS OBSCURUS*

G. CLIFF* and G. D. THURMAN
Oceanographic Research Institute, P.O. Box 10712, Marine Parade, 4056 Durban, Republic of South Africa

(*Received* 15 *August* 1983)

Abstract—1. Blood parameter changes caused by the stress of capture and simulated transport were studied in the juvenile dusky shark, *Carcharhinus obscurus*.

2. Plasma concentrations of potassium, calcium and magnesium ions, creatinine kinase, blood lactate and glucose levels and the partial pressure of carbon dioxide were elevated during stress.

3. The plasma bicarbonate ion concentration and blood pH declined.

4. The acidosis was evident from the onset of stress, minutes before blood lactate levels rose.

5. The sharks required a recovery period of approx 24 hr before most of the parameters had regained their prestress blood levels.

INTRODUCTION

It is acknowledged that sharks are among the most difficult of all fish to catch, transport alive and establish in captivity (Davies, 1964). Research on teleosts (Black, 1958) and elasmobranchs (Rasmussen and Rasmussen, 1967; Piiper and Baumgarten, 1969) has shown that the acid base balance of their blood can be severely disrupted by the formation of large amounts of lactic acid during hyperactivity. Martini (1974) found that marked changes in blood electrolyte concentrations occurred in the dogfish (*Squalus acanthias*) immediately after capture and following introduction to experimental pens.

We have encountered a high mortality in large pelagic sharks due to the trauma associated with capture and transport. These animals were required in preparation for tests to determine their responses to an electrical barrier designed as a shark repellent. Efforts were made to ascertain the possible causes of death and blood samples were taken from large sharks stressed during capture and transport to the Durban Aquarium. In view of the difficulties of catching and bleeding these animals without subjecting them to a considerable amount of stress, it was not possible to obtain blood values from 'normal, unstressed' sharks which could be compared with those taken from sharks close to death. Consequently, this study was undertaken to determine the effects of capture and transport (confinement) stress on the blood chemistry of the juvenile dusky shark, *Carcharhinus obscurus*. These sharks are abundant close inshore along the east coast of South Africa for several months in the year and are easily caught and bled to obtain values which closely approximate the normal, unstressed condition. The same blood parameters were again measured after the stress of capture and confinement to ascertain the recovery time.

This research is being extended to developing therapeutic measures aimed at reducing the deleterious effects of capture stress and thereby speeding up the recovery time (Cliff and Thurman, in preparation). In time the results may not only be used to provide healthy, large sharks for the electrical barrier experiments but to obtain such animals for display in aquaria.

MATERIALS AND METHODS

Shark capture

The sharks (standard length 63–75 cm, body mass 4.5–7.5 kg) were caught at sea on rod and line from a 6.6 m boat. Once a shark was hooked, it was boated immediately using a hand-net and placed on its back in a restraining board similar to that used by Jones and Geen (1974) and two cardiac blood samples (8 and 2 ml) taken. This procedure was completed in less than 2 min. Twenty sharks were sampled in this manner. It was considered reasonable to regard these values as "minimal stress" baseline values for the parameters under investigation.

Hyperactive period

In addition to the above, a further 12 sharks were left to struggle in the water for 10 min. A shark from this group would fight vigorously for the first 3 min, thereafter becoming progressively weaker so that during the final 3 min the shark was often motionless, appearing to be totally exhausted, with its tail gradually dropping so that the body was almost vertical in the water. Once netted, the shark resumed struggling but was easily restrained and bled.

Confinement period

In order to assess the effects of confinement occurring during transport, a third batch of sharks was boated after 10 min of hyperactivity and immediately placed in the boat's 3.1 × 0.7 m tank which was half filled with sea-water. The water was continually renewed using a submersible bilge pump with a pumping capacity of 8000 l per hr. No more than six sharks were held in the tank at any one time. Thirteen sharks were sampled after 30 min in the tank

*Present address: Natal Anti-Shark Measures Board, Private Bag 2, Umhlanga Rocks 4320, Republic of South Africa. Telephone: 511238.

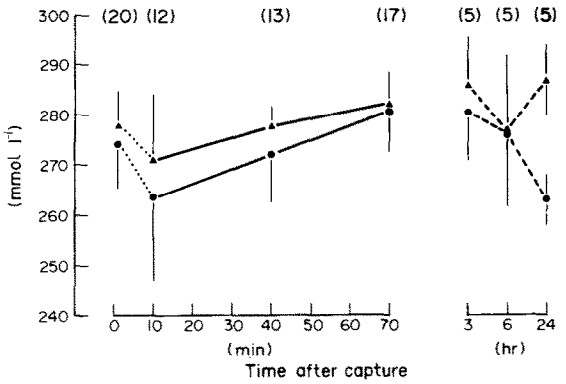

Fig. 1. Changes in venous plasma sodium (●) and chloride (▲) concentrations during hyperactivity (dotted line), and confinement (solid line) and after stress (dashed line) in the juvenile dusky shark, *C. obscurus*. Means and 95% confidence limits are plotted; the number of samples is given in parentheses.

(40 min after capture), and another 17 sharks were sampled after 60 min (70 min after capture). During the first 30 min of confinement, the sharks, although unable to swim freely and turn in one movement, were fairly active. Thereafter they were usually recumbent on the bottom of the tank, but would resume swimming when prodded. All the sharks were released alive at sea. Preliminary work showed that if confinement was extended much beyond 60 min all the specimens became extremely pale and sluggish and many showed signs of rigidity along the swimming muscles. It is unlikely that these animals would have survived. For this reason confinement was limited to a maximum of 60 min.

Post-stress period

To determine how quickly the animals recovered to minimum stress levels, a fourth group of five sharks was transported in sea-water to the laboratory after spending approx 30 min in the tank on the boat. This journey, a distance of 5 km, took 25 min, resulting in a total confinement period of no more than 60 min. In the laboratory the sharks were placed in a pool 5 m in diameter and 1.2 m deep, where they were able to swim and turn without bumping into the walls of the tank. Three blood samples were taken from each shark 3, 6 and 24 hr after capture. Water temperature in the pool remained fairly constant at 20°C during the experiments, due to a rapid exchange with the sea.

Blood sampling

The sharks were bled by means of cardiac puncture. No alternative source of venous or even arterial blood with such rapid and easy access was found. One 8 ml sample of blood was removed, 6 ml of which was transferred to a plain 10 ml evacuated tube. The remaining 2 ml was placed in a 3 ml tube containing 6 mg of potassium oxalate and 7.5 mg sodium fluoride for glucose and lactate analyses. An additional 1 ml of blood for pH and blood gas determinations was drawn into a 2.5 ml plastic disposable syringe whose dead space had been filled with liquid heparin (Pularin, 5000 units per ml). The syringe was agitated to ensure thorough mixing of the blood and anticoagulant and the needle bent to seal the syringe.

The samples were kept cool (5–10°C) and despatched to a commercial pathology laboratory within 2 hr for analysis.

Analytical procedures

Blood in the plain tubes was centrifuged at 1.5 *g* for 10 min. The sodium and potassium concentration of the plasma were determined using a Corning Ion Specific Electrode. Chloride analysis was undertaken using a Corning 125 Chloride Analyser and the coulometric titration method of Cotlove (1961). Magnesium was measured with a Varian 275 Atomic Absorption Spectrophotometer. Serum bicarbonate was measured with a Corning CO_2 Analyser.

Osmolality was measured with a Halbmikmicro Osmometer. Boehringer test kits were used for total and ionized calcium, creatinine kinase (CK) and lactate determinations. The plasma glucose concentration was determined by the glucose oxidase method of Kadish *et al.* (1968) using a Beckman Glucose Analyser.

pH, pCO_2 and pO_2 were measured with an Instrumentation Laboratory Model 431 pH Blood Gas Analyser, an instrument only able to carry out analyses at 37°C, which is approx 17°C higher than the body temperature of the sharks. In addition to this problem, we realise the limitations involved in using venous blood for critical acid–base studies. However, the aim of this research was to detect relative changes in the acid–base parameters occurring with stress, rather than to determine accurate acid–base levels.

RESULTS

Mean values of each blood parameter were calculated at every time interval during and after stress; the values were plotted together with the 95% confidence limits in Figs 1–9. The data, except that from sharks kept in the laboratory (post-stress period), were subjected to an analysis of variance test followed by a Duncan's multiple range test to examine the differences between pairs of means. Probabilities of less than 5% were considered significant.

Serum electrolytes

Sodium and chloride ions were present in the blood at concentrations in the region of 280 mmol l^{-1} (Fig. 1). There was no significant change in the levels of either of these ions throughout the period of stress.

Potassium, principally an intracellular cation, rose significantly during the hyperactive period from 3.3 to 5.3 mmol l^{-1} and again during confinement (Fig. 2). In the post-stress period the concentration remained high, but it had almost returned to the baseline level within 24 hr.

The concentration of total serum magnesium increased progressively with stress (Fig. 3). The levels recorded during confinement were significantly

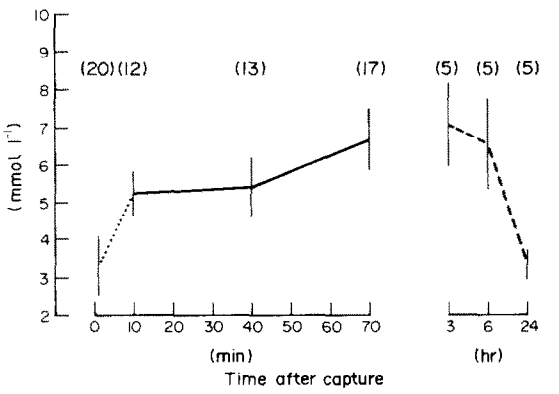

Fig. 2. Changes in venous plasma potassium concentration in the juvenile dusky shark, *C. obscurus* during and after stress. Other details are as in legend to Fig. 1.

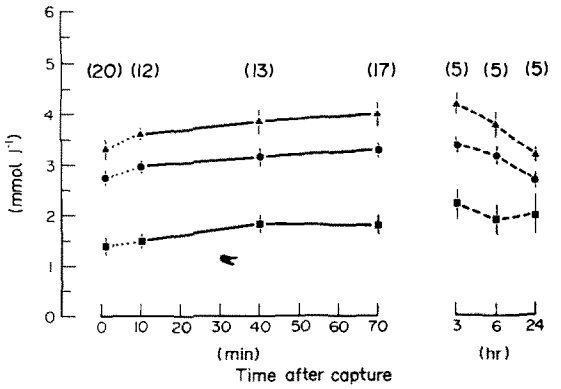

Fig. 3. Changes in venous plasma magnesium (■), total calcium (▲) and ionized calcium (●) concentrations in the juvenile dusky shark, *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

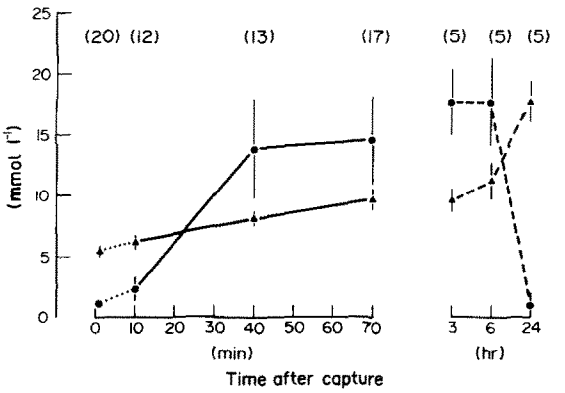

Fig. 5. Changes in venous blood lactate (●) and glucose (▲) concentrations in the juvenile dusky shark, *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

higher than the 1 and 10 min values. The concentration rose to 2 mmol l^{-1} and remained high during the post-stress period, though in one shark it had dropped to 1.4 mmol l^{-1} after 24 hr.

Both total and ionized serum calcium levels (Fig. 3) rose during the 10 min hyperactive period, followed by a more gradual increase during confinement. The 70 min value was significantly higher than those recorded at 1 and 10 min. Over 90% of the increase in total calcium was due to a rise in the ionized fraction, with the exception of the first 30 min of confinement when the bound calcium fraction accounted for 35% of the increase in total calcium. During the post-stress period the concentrations dropped after 6 hr and by 24 hr they were close to baseline values.

Creatinine kinase, lactate, glucose, osmolality

Serum levels of the intracellular enzyme, creatinine kinase, were extremely variable (Fig. 4). There was a sharp increase during hyperactivity. During the post-stress period concentrations remained high.

There was an enormous increase in the concentration of blood lactate from 1 to 15 mmol l^{-1} over the 70 min stress period (Fig. 5). A small insignificant rise after 10 min was followed by a significant increase during the first 30 min of confinement. In the initial stages of the post-stress period the lactate concentrations continued to rise. Some time after 6 hr they began to decline, approaching 1 mmol l^{-1} (the minimal stress value) within 24 hr.

A 77% increase in the concentration of blood glucose was recorded during the 70 min stress period (Fig. 5). Levels rose linearly during the first 70 min and remained constant at just under 10 mmol l^{-1} until 3 hr after capture. The 40 min sample was significantly different from the 1, 10 and 70 min samples. During the post-stress period the concentration started to rise after 3 hr, exceeding 15 mmol l^{-1} at 24 hr.

The serum osmolality rose from 1027 to 1089 mmol l^{-1} in 70 min (Fig. 6); this increase was greatest during the first 10 min. The 40 and 70 min values were significantly higher than the 1 min value. During the post-stress period the osmolality dropped, attaining 1020 mmol l^{-1} after 24 hr.

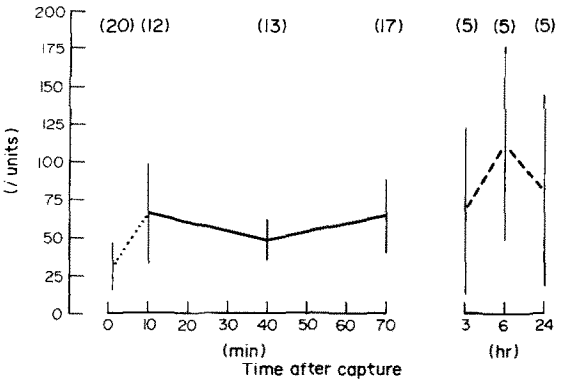

Fig. 4. Changes in venous plasma creatinine kinase concentration in the juvenile dusty shark, *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

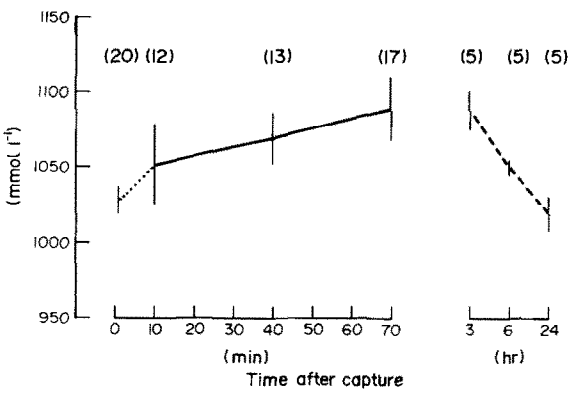

Fig. 6. Changes in venous blood osmolality in the juvenile dusky shark, *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

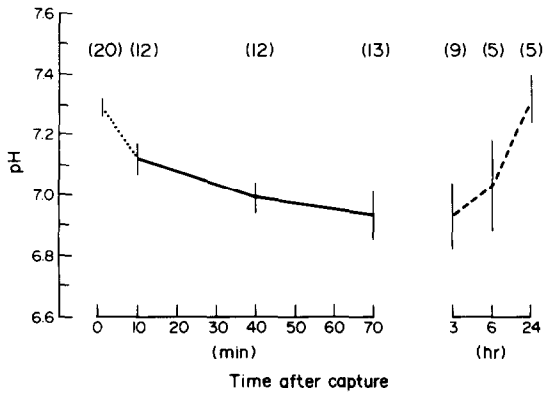

Fig. 7. Changes in venous blood pH in the juvenile dusky shark, *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

Acid–base balance

During the first 10 min the pH declined sharply from 7.29 to 7.12 (Fig. 7). This was followed by a smaller but still significant drop to 6.99 after the first 30 min of confinement. A pH of 6.93 was recorded at 70 min and again at 3 hr, thereafter the pH started to rise and reached the minimal stress level by 24 hr.

The concentration of serum bicarbonate ions dropped sharply after 10 min of hyperactivity from 4.6 to 3.6 mmol l^{-1} (Fig. 8). Although the decline during confinement was slower, these levels were significantly lower than that recorded 1 min after capture. The concentration, which continued to fall during the early post-stress period, started rising after 3 hr. It attained 4.0 mmol l^{-1} by 24 hr, which was still below the baseline value.

There were two significant changes in the partial pressure of carbon dioxide (Fig. 9): a 12% increase in the first 10 min, followed by a rise of similar magnitude during the first 30 min of confinement. Thereafter the pCO_2 remained constant. After 6 hr the pCO_2 dropped below 1.7 kPa to values which were markedly lower than the minimal stress levels.

There were no significant changes in the partial pressure of oxygen (Fig. 9). The large variability encountered is not unusual when sampling venous blood. The pCO_2 appeared to drop after hyperactivity.

DISCUSSION

Acid–base balance

On of the physiological effects of severe exercise in animals, including fish, is an increasing acidosis caused by the build-up of metabolic end products, primarily lactic acid (Love, 1970). Bennett (1978) has attributed the rise in lactic acid to the extremely low aerobic capacities of lower vertebrates when compared to birds and mammals. This means that fish, amphibians and reptiles must rely heavily on anaerobic metabolism to support vigorous activity. At physiological pH, lactic acid is virtually dissociated into lactate and hydrogen ions (Albers, 1970) to the detriment of the acid–base balance of the blood and the muscle cells in which it is produced.

In this study, the venous blood pH of *C. obscurus* dropped from 7.29 to 7.12 after 10 min of hyperactivity, representing a 57% increase in the H^+ concentration, accompanied by a substantial decrease in HCO_3^- concentration. During this period there was a relatively small rise in blood lactate in comparison to the increases which took place during confinement. The rapid decline in venous blood pH during struggling may be attributed largely to an elevation in pCO_2. Wood *et al.* (1977) report similar findings in the flounder, *Platichthys stellatus*. The pCO_2 of *C. obscurus* sampled after several days in the laboratory pool was in the region of 1.5 kPa, while the baseline value in Fig. 9 was 2.2 kPa. At sea, a delay of nearly 1 min from the time the shark was hooked until the time it was netted would account for this difference in pCO_2 levels. As a result, the actual increase in venous blood pCO_2 after 10 min struggling appears to be far greater than that shown in Fig. 9. The rise in pCO_2 during hyperactivity is the result of an increased CO_2 production by the muscle cells, which may be accentuated by a disruption of ventilation and blood circulation, particularly while the shark is out of the water, thereby inhibiting the off-loading of CO_2 at the gills. These factors would also result in a drop in pO_2 during struggling.

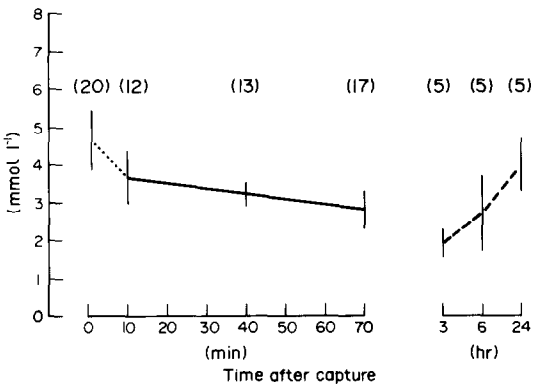

Fig. 8. Changes in venous plasma bicarbonate concentration in the juvenile dusky shark. *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

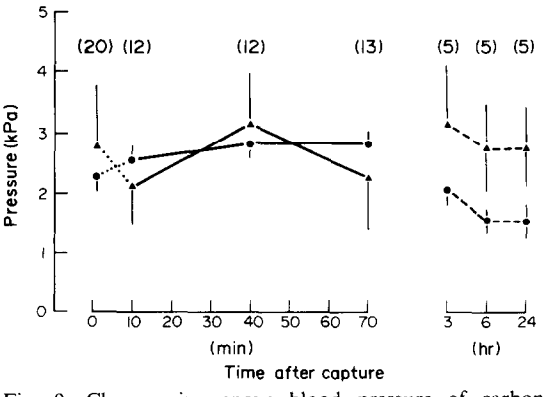

Fig. 9. Changes in venous blood pressure of carbon dioxide (●) and oxygen (▲) in the juvenile dusky shark, *C. obscurus*, during and after stress. Other details are as in legend to Fig. 1.

The pCO_2 continued to rise during the initial 30 min of confinement, although the associated drop in pH from 7.12 to 6.98 was, probably, due mainly to a release of lactic acid into the blood as it coincides with a large increase in blood lactate during this period. Thereafter, the pCO_2 remained constant and any decline in pH can be attributed to the continued entry of large amounts of lactic acid into the blood from the muscles.

Three hours after capture the blood pH had begun to rise, however, the lactate concentration continued to increase and only some time after 6 hr did the latter start to drop. There have been several other reports of a discrepancy between blood pH and lactate levels in the blood of fish after exhausting capacity (Piiper *et al.*, 1972; Wood *et al.*, 1977; Wardle, 1978; Beggs *et al.*, 1980; Wood *et al.*, 1983). Piiper *et al.* (1972) suggest that some of the H^+ ions obligatorily produced with lactate may be retained and buffered in the tissue of the dogfish, *Scyliorhinus stellaris*. The apparent branchial uptake of bicarbonate from the external environment may improve the buffering capacity of the blood of this species (Holeton and Heisler, 1978). The HCO_3^- concentration in *C. obscurus* rarely exceeded 6 mmol l^{-1}, which is extremely low in comparison to mammalian levels of 25 mmol l^{-1} (Ganong, 1973). Consequently, the bicarbonate concentrations in these sharks are rapidly exhausted, thereby providing a limited buffering power. The role of non-bicarbonate buffers should therefore be examined, particularly in view of the large intra- and extracellular amounts of urea, amino acids and trimethylamine oxide found in elasmobranchs. In mammals, metabolic acidosis results in increased production of ammonia from amino acids which can take up the excess H^+ ions (Harper, 1973).

Of all the blood parameters measured, pH appears to be the most accurate reflection of the degree of stress in *C. obscurus*. The following significant correlations ($P < 0.05$) were found between pH and other parameters: K^+ ($r = -0.61$); Mg ($r = -0.57$); Ca ($r = -0.50$); lactate ($r = -0.67$); glucose ($r = -0.76$); pCO_2 ($r = -0.59$); HCO_3^- ($r = 0.64$).

Serum electrolytes

Capture and confinement disrupted blood levels of other cations in addition to hydrogen. Concentrations of potassium, calcium and magnesium ions rose markedly from the onset of stress. This appears to be due to leakage from the muscle cells caused by a disturbance in cellular function during hyperactivity and a loss of cell membrane integrity through increasing lactacidosis. Martini (1974) attributed part of the rise in plasma potassium ions in *S. acanthias* following capture to haemolysis, which may have been caused by high lactate levels. As there was no evidence of haemolysis in this study, we suspect that the serum potassium originated from the muscle cells. In mammals, lactacidosis, or any other condition causing cellular disruption, is known to result in hyperkalaemia. Once blood potassium levels approach 7 mmol l^{-1} myocardial function is disrupted, causing bradycardia and a decrease in cardiac output, thereby potentiating anaerobic metabolism in the muscles and increasing lactic acid production (Guyton, 1971).

Few papers have been concerned with effects of handling or similar stress on the divalent cation balance of fish. Soivio and Oikari (1976) report that there were marked changes in the concentrations of plasma potassium, calcium and magnesium in the teleost, *Esox lucius*, after handling. These increases generally only lasted 4 hr. Thereafter, levels dropped below the initial values which were restored after 2 days.

The rise in plasma calcium in *C. obscurus* is due largely to an increase in the ionized or active fraction. However, this was not at the expense of the bound calcium which also increased slightly with time. In mammals an increase in ionized calcium in the plasma appears to be a natural consequence of acidosis (Stogdale, 1981). The changes in extracellular levels of calcium and magnesium may impair muscle contraction and neuromuscular nerve transmission.

Plasma sodium and chloride concentrations remained fairly constant during stress, which is in keeping with the results of studies on teleosts (Soivio and Oikari, 1976; Beggs *et al.*, 1980).

Creatinine kinase, blood glucose and osmolality

The increase in plasma levels of the intracellular enzyme, creatinine kinase, is also indicative of increased muscle cell permeability or, in its extreme form, loss of cell integrity. An equally large variation in plasma CK levels has also been recorded in stressed zebras (Hofmeyer *et al.*, 1973) and birds (Henschel and Louw, 1978).

A rise in blood glucose is recognized as a response to stress in a broad spectrum of animals including elasmobranchs (Mazeaud *et al.*, 1977). Soivio and Oikari (1976) cite Scott (1921) who found that removing the dogfish *Mustelus canis* from water for 80 sec increased the blood sugar from 70 to 170 mg% (3.9–9.5 mmol l^{-1}) in 2.5 min. This rise is believed to be induced by the secretion of catecholamines (either adrenalin or noradrenalin) into the blood (Love, 1970). De Roos and De Roos (1978) found that a single injection of adrenalin stimulated a rise in blood glucose which lasted 24 hr, attaining a maximal 72% increase after 3 hr. Patent (1970) found that the elevated levels lasted for as long as 48 hr.

Muscle glycogen is rapidly depleted during intense activity and appears to be the principal source of lactic acid formed during anaerobic metabolism in lower vertebrates (Bennett, 1978). As suggested by Wardle (1978), the elevation in blood glucose may form part of a restorative process in which the glucose is mobilized from the liver glycogen stores. It enters the muscle cells, supplying both raw material and energy for the rebuilding of muscle glycogen. Blood glucose levels may also be elevated by corticosteroids released into the blood following stressful environmental conditions (Hille, 1982). Patent (1970) found that exogenous cortisol and corticosterone induced hyperglycemia in *S. acanthias*. In this study, the reason for the enormous increase in blood glucose after 6 hr is not certain, however, it did coincide with the drop in blood lactate levels. Once anaerobic metabolism is resumed it is possible that the lactate is converted to glucose. Murdaugh *et al.* (1965) report that there is little excretion of lactate at the gills by

S. acanthias. Bennett (1978) states that the fate of the lactate formed during strenuous activity in the lower vertebrates is largely unknown. In mammals, the lactic acid can either be reconverted to glucose or used directly for energy in the citric acid cycle (Guyton, 1971).

The blood glucose levels of two *C. obscurus* which died soon after arrival at the laboratory were found to be extremely low (5.7 and 2.9 mmol l^{-1}). It is tempting to attribute the cause of death to an inability to mobilize the necessary sugars needed as an energy source by muscle tissue. The blood glucose levels of sharks held in the recovery pool for 3–4 days were found to have returned to the minimal stress levels of 5 mmol l^{-1}.

There was a 6% increase in blood osmolality during stress. In view of the exceptionally high values found in elasmobranchs, we do not regard this increase as having a detrimental effect on juvenile *C. obscurus*.

Clinical observations

We observed that the sharks turned pale during confinement; the change in skin colour becoming more marked with time. On recovery, the sharks regained their normal grey colour within 1 hr. Many fish are known to respond in this manner when experiencing stress. This phenomenon may be attributed to the effect of increased levels of catecholamines on the melanocytes (Mazeaud *et al.*, 1977) and on the peripheral blood circulation causing vasoconstriction.

We noted an increase in trunk muscle rigidity following lengthy confinement. Martini (1974) observed stiffening in dogfish, *S. acanthias*, which had experienced considerable stress during capture. We have also encountered tetany in adolescent *C. obscurus* and *C. leucas* after a long period of hyperactivity and confinement. On release, the sharks appeared to be too exhausted to swim and stiffened from the anterior muscles caudally. This tetany may be caused by rising levels of extracellular potassium which lower the threshold levels for nerve impulse generation to such an extent that there is almost continual stimulation of the muscles.

Cause of death

The cause of death in *C. obscurus* following capture and confinement was not conclusively identified, however, the effects of acidosis and to a lesser extent hyperkalaemia appear to be the most detrimental to the survival of the sharks. Wood *et al.* (1983) discount the hypothesis that "post-exercise mortality in fish is due to excessive lactic acid accumulation in the blood." It would appear that over 80% of the lactate and hydrogen ions produced by the exercising white muscle tissues of the rainbow trout, *Salmo gairdneri* is never released into the blood. This has led the authors to suggest that intracellular acidosis may be the critical toxic event. Nevertheless, irrespective of the locality, the aims of any corrective therapy must be to alleviate the disruption in the acid–base balance.

Recovery

It is interesting to note that the effects of stress on the blood chemistry of *C. obscurus* were not reversed once the stress was removed, but continued to manifest themselves and in some cases deteriorated during the first few hours of the post-stress period. We believe that the delay in the recovery of these animals may be attributed to an impairment of blood circulation during confinement. Once the normal swimming motion is resumed in the laboratory there is a perfusion and flushing of the muscle tissue with fresh blood and it is only then that those substances which have been sequestered in the tissues and extracellular fluids enter the systemic circulation, resulting in substantial and possibly lethal increases in their concentrations in the blood entering the heart.

Extended sampling over a 24 hr period revealed that only plasma glucose and magnesium had not returned to prestress levels by the end of this period. From this it can be concluded that a recovery time of at least 24 hr is needed before juvenile *C. obscurus* can be used for physiological experimentation, unless supportive therapy is developed to minimize the various pathological and physiological changes occurring in the stressed animals.

Acknowledgements—We thank the heads and staff of the laboratories where the analyses were undertaken. Rudy van der Elst, Colin Sapsford, Dr Mike Schleyer, Prof. J van Heerden, Dr Daan Goosen and Walter Deppe gave advice in the preparation of this manuscript. Staff of the Natal Anti Shark Measures Board assisted in the field. The study was funded by the South African National Committee for Oceanographic Research.

REFERENCES

Albers C. (1970) Acid–base balance. In *Fish Physiology* (Edited by Hoar W. S. and Randall D. J.), Vol. IV, pp. 173–208. Academic Press, New York.

Beggs G. L., Holeton G. F. and Crossman E. J. (1980) Some physiological consequences of angling stress in muskellunge. *Esox masquinongy* Mitchill. *J. Fish Biol.* **17,** 649–659.

Bennett A. F. (1978) Activity metabolism of the lower vertebrates. *A. Rev. Physiol.* **400,** 447–469.

Black E. C. (1958) Hyperactivity as a lethal factor in fish. *J. Fish. Res. Bd Can.* **15,** 573–586.

Cotlove E. (1961) Chloride measurements using coulometric titration. In *Standard Methods of Clinical Chemistry* (Edited by Seligson D.), Vol. 3, pp. 81–85. Academic Press, New York.

Davies D. H. (1964) *About Sharks and Shark Attack*. Shuter and Shooter, Pietermaritzburg, South Africa.

De Roos R. and De Roos, C. (1978) Elevation of plasma glucose levels by catecholamines in elasmobranch fish. *Gen. comp. Endocr.* **34,** 447–452.

Ganong W. F. (1973) *Review of Medical Physiology*, 6th edn. Lange Medical Publications, Los Altos, California.

Guyton A. C. (1971) *Basic Human Physiology: Normal Function and Mechanism of Disease*. W. B. Saunders Company, Philadelphia.

Harper H. A. (1973) *Review of Physiological Chemistry*, 14th edn. Lange Medical Publications, Los Altos, California.

Henschel J. R. and Louw G. N. (1978) Capture stress, metabolic acidosis and hyperthermia in birds. *S. Afr. J. Sci.* **74,** 305–306.

Hille S. (1982) A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri* Rich. *J. Fish Biol.* **20,** 535–569.

Hofmeyer J. M., Louw G. N. and Du Preez, S. (1973) Incipient capture myopathy as revealed by blood chemistry of chased zebras. *Madoqua* **1,** 45–50.

Holeton G. and Heisler N. (1978) Acid–base regulation by bicarbonate exchange in the gills after exhausting activity in the larger spotted dogfish, *Scyliorhinus stellaris*. *Physiologist* **21,** 56.

Jones B. C. and Geen G. H. (1974) Small shark restraining apparatus. *Prog. Fish Cult.* **36,** 115.

Kadish A. H., Little R. L. and Sternberg J. C. (1968) The determination of glucose by measurement of the rate of oxygen consumption. *Clin. Chem.* **14,** 116–119.

Love R. M. (1970) *The Chemical Biology of Fishes*. Academic Press, London.

Martini F. H. (1974) Effects of capture and fasting confinement on an elasmobranch, *Squalus acanthias*. Unpublished Ph.D thesis, Cornell University, New York.

Mazeaud M. M. Mazeaud F. and Donaldson E. M. (1977) Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* **106,** 201–212.

Murdaugh H. V., Jr., Robin E. D., Theodore J. and Drewry W. (1965) Studies of lactate metabolism in *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **5,** 30–31.

Patent G. J. (1970) Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch (*Squalus acanthias*) and a holocephalan (*Hydrolagus colliei*). *Gen. comp. Endocr.* **14,** 215–242.

Piiper J. and Baumgarten D. (1969) Blood lactate and acid–base balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. *Publ. Staz. Zool. Napoli* **37,** 84–94.

Piiper J., Meyer M. and Drees F. (1972) Hydrogen ion balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. *Resp. Physiol.* **16,** 290–303.

Rasmussen R. A. and Rasmussen L. E. (1967) Some observations on the protein and enzyme levels and fractions in normal and stressed elasmobranchs. *Trans. N.Y. Acad. Sci.* **29,** 397–413.

Soivio A. and Oikari A. (1976) Haematological effects of stress on a teleost, *Esox lucius* L. *J. Fish Biol.* **8,** 397–411.

Stogdale L. (1981) Correlation of changes in blood chemistry with pathological changes in the animal's body: II. Electrolytes, kidney function tests, serum enzymes, and liver function tests. *J. S. Afr. Vet. Assoc.* **52,** 165–164.

Wardle C. S. (1978) Non-release of lactic acid from anaerobic swimming muscle of plaice *Pleuronectes platessa* L.: A stress reaction. *J. exp. Biol.* **77,** 141–155.

Wood C. M., McMahon B. R. and McDonald D. G. (1977) An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. *J. exp. Biol.* **69,** 173–185.

Wood C. M., Turner J. D. and Graham M. S. (1983) Why do fish die after severe exercise? *J. Fish Biol.* **22,** 189–201.