STUDIES ON THE OSMOTIC AND IONIC REGULATION OF THE STRIPED DOGFISH PORODERMA AFRICANUM (GMELIN)

by

Geoffrey P. Haywood

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The South African striped dogfish

<u>Poroderma</u> <u>africanum</u> (Gmelin).

AUTHOR'S NOTE

All research reported in this thesis is original and has been arranged for publication as a series of scientific papers, in addition to forming the main contribution to the thesis, with the prior knowledge and consent of my supervisor, Professor A.C. Brown, and the Head of Department, Professor J.H. Day.

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SUMMARY

Under laboratory conditions, it has been found that the striped dogfish Poroderma africanum (Gmelin) will tolerate a wide range of salinities - from approximately 18.0 to 47.5‰. Over this range, serum chloride was found to vary from 200 mM/1, in the most dilute medium, up to 315 mM/1, at the highest salinity. Blood osmolarity paralleled the isosmotic line over most of the salinity range, being 40 to 60 mOsm/1 above the line for normally-fed animals. Animals which had been fed only once a month, however, showed a marked reduction in serum urea concentration which resulted in reduced serum osmolarity. Such animals displayed hypo-osmotic regulation and since the degree of this was found to increase with starvation time, it is suggested that animals fed at intermediate times up to once a month would show different degrees of hypo-osmotic regulation.

Later measurements of serum sodium showed, interestingly, that the blood sodium level was lower than the blood chloride level for fish in normal sea water (467 mM Na/1, 550 mM Cl/1 1020 - 1040 mOsm/1). In such a medium, the mean serum chloride was found to be 261 mM/1, sodium was 238 mM/1, urea was about 460 mM/1 for normally-fed fish and about 410 mM/1 for underfed ones, and serum osmolarity averaged about 1080 mOsm/1 for normally-fed fish and about 980 mOsm/1 for underfed ones.

Over a similar range of external salinities, however, the change in serum sodium did not parallel that of serum chloride concentration. At about 107% sea water, the increase in blood sodium concentration with increase in external salinity became quite marked so that at higher salinities serum equalled and then exceeded the serum

chloride level. A brief investigation of serum potassium levels showed that this only varied from 3.0 to 4.7 mM/l, for fish in dilute and hyper-saline media.

Using radio-isotopes, the turnover rates of sodium, chloride, water and urea were measured, for fish in normal sea water, and were found to be 0.24, 2.47, 97 and 0.08%/h respectively. Turnover rate measurements at different salinities showed that values for chloride and water were maximal in normal sea water and showed decline with variation away from this salinity. A pronounced salinity effect was shown for urea turnover, with increased rate at higher salinity. The exchangeable ionic space was measured as chloride space and was found to be 34.4% body volume or 32.4 ml/100g fresh weight.

Blood haematocrits have shown that normally-fed dogfish, which do not drink the medium, experience dilution of the blood when exposed to a medium dilution of about 100 mOsm/l suddenly - and similarly suffer blood concentration, when exposed to a corresponding rise in the external osmolarity. On remaining in the new medium, blood values have been shown to return to normal within 7 days - indicating the re-attainment of water balance by the animals within this period. It has been estimated that the major water flux responsible for this effect on the blood, probably occurs at the gills. Hypo-osmotic animals, which were found to drink the medium, experienced greater effects of salinity change upon blood concentration and took longer to attain water balance after transfer to the new medium. Both the radio-isotopes Chloride-36 and Sodium-22 were shown to be lost at the gills, against the concentration gradient. Dogfish with rectal glands and urinary systems surgically closed were found to osmoregulate satisfactorily over a limited time period, with only the gills as osmoregulatory organs. Together with this, histological silver staining of gill tissue from fish under several different experimental conditions, showed intra-cellular chloride deposits in cells of the secondary lamellae and suggested the possibility of chloride transport by the gills of this species.

In an investigation into the roles played by the kidneys and rectal gland, in the osmotic and ionic regulation of this species, it was found that fish with inoperative rectal glands showed only slight rises in blood sodium and chloride levels, over a 2-week period, as compared with control animals. Salt-loading of both fish with inoperative rectal glands and normal animals showed that in both cases blood salt levels returned to normal within 7 h of salt injection, but comparison between the two groups suggested that the rectal gland might regulate chloride more than sodium. A composite fluid, assumed to be composed of urine and rectal gland fluid, was collected from normal animals and, from analysis of this, the average cloacal salt loss rate was estimated. Urine was also collected separately from normal fish and its contribution to the salt loss was estimated.

Histochemical examination, including the chloride test, was also carried out on rectal gland tissue from normal and salt-loaded fish, in different salinities. A general radial arrangement of secretory tubules was apparent in the rectal gland of this species, although, in the lower regions of the gland, the glandular parenchyma was divided up into lobules, so that the radial arrangement was only clear in upper glandular tissue. The chloride test showed chloride deposits in cells of the secretory epithelium, which showed a marked resemblance to the intracellular deposits observed in branchial tissue of this species. It was concluded that specific chloride transport by cells might occur in the tubules of the rectal gland, providing a similar ionic regulatory mechanism to that observed in the gills.

(During the course of this work, specimens of the rabbit fish *Callorhinchus capensis* (Dumeril) were caught live from a local source and preliminary investigations of blood composition were carried out. A short note on initial findings is presented in the Appendices.)

CHAPTER 1

INTRODUCTION

This thesis presents ideas, techniques, results and conclusions from a 3-year research project regarding the osmotic and ionic regulation of the South African striped dogfish Poroderma africanum (Gmelin). The experiments were devised early in 1971 but due to the time required for personally constructing equipment and facilities and for modifying and equiping the laboratory and aquaria with the necessary apparatus, actual experimentation and subsequent obtaining of results was not possible before November 1971.

The main theme of the research was the investigation of the major body fluids and their relationship to external conditions, in a local elasmobranch species. The topic has been extensively examined in some overseas countries but so far very little physiological work on the osmoregulation of marine elasmobranchs has been carried out in the South African region. The species chosen was also considered interesting in view of its common occurrence around the Cape (at the time of commencement of the work) and the fact that it seemed to be relatively limited in its distribution around the African coast, although in appearance it resembles the Scyliorhinid dogfishes widely distributed around overseas coasts.

The findings and conclusions from this work form the basis of 6 scientific papers which in turn form the major part of this thesis. These papers have been submitted to scientific journals and are in various stages of publication, as indicated in the relevant sections.

It was shown by early workers (Scott, 1913; Chaisson, 1930; Margaria, 1931) that changes in the external salinity produce effects upon the electrolyte balance in the blood of marine elasmobranchs. They showed that when an elasmobranch is placed into diluted sea water, the overall osmolarity of its blood falls and, conversely, rises when the fish is exposed to concentration of the medium. They deduced that the change in blood osmolarity was produced by a change in the concentration of the various ionic and osmotic components.

In 1930, Smith suggested that osmoregulation in marine teleosts was largely effected by drinking of the medium and extraction and excretion of salts by the gills. In this way they were able to maintain their low blood salt level (about one-half that of sea water) and at the same time compensate for the constant tendancy to lose water to the exterior by ex-osmosis. Later work (Smith, 1931) suggested that this was not the case for marine elasmobranchs, and he was not able to determine any appreciable ingestion of the medium by these animals.

Keys and Willmer (1932) showed the presence of 'chloride cells' in the gills of marine teleosts, which added weight to the hypothesis of branchial salt regulation, but again they were unable to detect such cells in the gills of the dogfish <u>Scyliorhinus</u> - the only elasmobranch they examined.

Although this had been detected by earlier workers, Smith (1936) and later Kempton (1953) showed that elasmobranchs retained urea in the tissues and body fluids, to an appreciable extent, and by so doing, were able to maintain a blood osmolarity equal to or slightly in excess of the external medium. This obviated the osmotic deficit between body fluids and sea water experienced by marine teleosts, although there still existed the matter of salt imbalance.

At this stage it was not really understood how the marine elasmobranchs maintained salt balance since they had not been found to produce hypertonic urine - which would be necessary, were the kidneys to effect ionic regulation - and they did not appear to employ branchial salt regulation, as was the case for marine teleosts. It was only in 1960, when Burger and Hess postulated the function of the elasmobranch rectal gland as being a salt gland, that a possible solution to the enigma was found. Later work (Burger, 1962, 1965) further supported the earlier suggestion and, until recently, it had become generally accepted that the rectal gland in marine elasmobranchs acts as a salt gland and is the major site of ionic regulation, since it is able to secrete a very concentrated saline solution.

Since then, various suggestions have been put forward regarding urea reabsorption techniques in marine elasmobranchs (Boylan, 1967; 1972; Schmidt-Nielsen, 1972) and considerable experimentation on the general osmoregulation and turnover of various ions and osmotic components has been performed (Burger and Tosteson, 1966; Maetz and Lahlou, 1966; Chan _et al_., 1967 Price and Creaser, 1967; Goldstein _et al_., 1968; Goldstein and Forster, 1971; Payan and Maetz, 1970, 1971, 1973; Carrier and Evans, 1972, 1973; Payan _et al_., 1973; de Vlaming and Sage, 1973; etc.)

The first indication that perhaps things were not as simple as Burger and Hess suggested, can be traced back to 1961, when Doyle and Gorecki, upon investigation with the electron microscope, found 'chloride cells' in the gill tissue of the marine elasmobranch

Urobatis. Later work by Maetz and Lahlou (1966) suggested that branchial salt regulation in the dogfish *Scyliorhinus* was not only apparent, but seemed to be more important than the regulatory action of either the kidneys or the rectal gland. Indeed their results suggested that the ionic regulation effected by the rectal gland was only minimal by comparison with that of the gills. These findings were later supported by the work of Payan and Maetz (1973) who reported 'chloride cells' as being present in *Scyliorhinus canicula*. Payan *et al*. (1973) also showed that branchial urea exchange was considerably greater than that via the urine.

The overall picture, regarding osmoregulation in marine elasmobranchs, is therefore no longer a simple one, and in the light of these previous findings of other workers it was decided to conduct similar investigations on a local species.

It should be noted that the studies reported here have all been carried out under laboratory conditions and whilst these have been made as near to field conditions as was practically possible, factors such as stress and artificial illumination probably affect the animals to an unknown degree. However, this reservation is true of the vast majority of physiological work carried out on any species.

CHAPTER 2

NOTES ON THE ECOLOGY AND FEEDING OF PORODERMA AFRICANUM (GMELIN)

Striped dogfish, known locally as "Pyjama sharks", are found on both sides of the Cape Peninsula, on the west coast as well as in False Bay.

Very little is known about the ecological distribution of the species. Smith (1965) has reported these dogfish as occurring right round the South African coast, from False Bay to Malagasi, and in water depths ranging from low tide to 80 m. Since many animals were caught for this research, from the west coast, it is possible to extend their distribution a little further than that reported by Smith. Nevertheless, examination of all pertinent fishing records held by the Zoology Department (Ecology files), U.C.T. and by the South African Museum, has failed to show any records of this species in estuaries or brackish water and it must therefore be concluded that Poroderma africanum is very much a stenohaline animal, with regard to its general distribution. Within False Bay, maximum annual salinity variation appears to be only from 35.00‰ to 35.28‰ (Atkins, 1970), which indicates that animals in this environment are not exposed to greatly differing salinities within the bay, especially when it is realised that these figures include all variations between surface and 30 m.

The great majority of animals used in the present research were collected by hand whilst SCUBA diving, and it was possible to make limited observations on the feeding of animals and the type of habitat chosen by the species. Observations have shown that the species is gregarious and many animals may be found in crevices and large holes under rocks. They are rarely encountered over sandy bottoms but seem to prefer a rocky substrate, especially where kelp and sea urchins are abundant. Water temperature does not seem to be a critical factor, provided it is below 20°C. Animals have been recovered from the west coast in water of about 9°C but also from False Bay, in water of about 16°C. As the water temperature approaches 20°C the animals become more difficult to find in water less than 10 m depth and fish have never been observed whilst diving in water above 20°C. In colder water, animals have been recovered from depths between 5 and 20 m. It may well be that during the summer (January to March), when the temperature of False Bay water rises, the dogfish move out into cooler deeper water.

All collections were made during the day and the above observations are therefore related to day-time behaviour. Reports by other divers have suggested that the species may be a nocturnal feeder, coming out of the crevices at dusk to forage for food, although animals have occasionally been observed to feed in the day-time. By comparison, laboratory fish do not appear to differentiate between day and night, with regard to feeding, and will feed satisfactorily during the day. However, a great many fishes, including elasmobranchs, are known to feed mainly at dawn and dusk.

Stomach content examination has indicated that the food of _Poroderma_ _africanum_ includes crayfish, molluscs such as _Donax_ and _Bullia_, and various small teleosts, but with a definite preference for cephalopods such as octopus and small cuttlefish. The release of cephalopod body fluids into sea water has been observed to initiate a typical elasmobranch 'feeding frenzy', during which several dogfish may attack the same piece of food. Indeed, larger animals have been observed to engulf smaller dogfish which have attempted to swim away with a large piece of food. The smaller animal is usually released after a while and appears unharmed. This phenomenon has been observed both in the field and under laboratory conditions.

The species is sluggish in its overall activity and seems disposed to take dying or damaged animal food rather than stalk active prey. In view of the gregarious nature of the species, it is envisaged that competition for food may well occur between animals of the same crevice or other micro-habitat.

CHAPTER 3

MAINTENANCE OF ANIMALS IN THE LABORATORY

At the onset of the research it was realised that a stock tank would be necessary for keeping live fish prior to experimentation. Furthermore, since the fish were to be kept over periods of up to 3 months, the stock tank would have to be supplied with running sea water, preferably filtered and sterilized.

There already existed, in the Zoology Department of the University, 2 adjoining thermostatically-controlled aquarium rooms, in one of which was a two-tier tank system, comprised of a pair of large stone tanks, each measuring approximately 2m x 2m x 1m, the upper tank built with its base about 1m above the top of the lower one. No sterilizing or filtering mechanism was present and indeed the tanks did not appear to have been used for a considerable length of time.

It was decided to use this existing system, with considerable modification, to supply filtered, sterilized sea water to a large stock tank in the adjacent aquarium room. A 2m x 1.5m x 0.5m glass fibre tank was set up on a pre-existing plinth in the adjacent room, such that it was at a height midway between that of the lower and upper tank of the two-tier system next door.

After much trial and error, the following system was used (with later minor modifications) for the maintenance of clean, filtered sea water in the two-tier system. Sea water was collected by truck and tank from Three Anchor Bay, Greenpoint and was transferred to the two tanks of the system. The working capacities of the upper and lower tanks were approximately 3000 and 2300 litres respectively. The lower tank formed a settling tank and from here the water was pumped up to a diatomaceous-earth filter, mounted above the upper tank. After being forced through the filter, the sea water passed through a distribution sprinkler which sprayed it evenly across one end of a 1.5 m long Perspex trough, passing under a bank of 4 1m-long mercury vapour ultraviolet (UV) fluorescent tubes, each of 30 watt rating. The tubes were positioned to hang approximately 1.5 cm above the water surface; the flow rate along the 40 cm wide trough being regulated to approximately 10 cm/second - producing a water depth of about 0.5 to 0.75 cm in the trough. From this trough the water dropped into the upper tank, where it formed the reservoir to supply stock tanks. A supply pipe ran through the wall between the 2 adjoining aquaria to supply the glass fibre stock tank next door, in which animals were kept. The system was designed to supply sea water to several other stock and display tanks and several connecting pipes and take-off points were coupled into this pipe once the system became operational. A second glass fibre tank was set up, for keeping dogfish, at a later stage, both tanks being positioned at a suitable height half way between that of the upper and lower tank of the adjacent two-tier system. The level of sea water in stock and display tanks was maintained by overflow return pipes joining and running back through the wall and returning sea water via a coarse filter into the settling tank of the two-tier system once again. The coarse filter was put into this return system to strain out any uneaten food or other particulate material from animals and therefore reducing contamination of water in the settling tank. The filter consisted of fine-mesh nylon gauze, which was periodically cleaned. From the settling tank, sea water was pumped up through the filter and ultraviolet sterilizing unit and into the upper tank, to complete the cycle. Due to the size of the lower tank, sufficient time was allowed for settling out of sediment, before the water was pumped up into the upper tank again.

Due to the very fine particulate size of the diatomaceous earth, the filter of the two-tier system was found to become blocked after 2 weeks, requiring regular changing of the earth. A by-pass pipe running between the pump and the ultraviolet sterilizing unit was put into the system so that the filter could be changed without stopping the pump but later the diatomaceous earth was replaced by activated charcoal in muslin bags. The inner material bag, designed to accommodate the diatomaceous earth, was removed to allow sufficient space for several bags of charcoal. This way it was possible to make changes of filter material only every 4 weeks or so.

The volume of water in the two-tier system was constantly checked. The sea water level in the lower tank was kept up to a fixed mark, by periodic additions of deionized water and the pump was equipped with an automatic float-operated mercury cut-out switch to prevent the pump ever running dry, in the event of any severe reduction in the level of the lower tank. The water level of the upper tank was maintained constant by means of a wide overflow pipe running back down through the floor of the upper tank to the lower settling tank. In order to ensure that this was always operating, it was necessary to pump water up into the upper tank at a faster rate than water was being drawn off to stock tanks. As

more display tanks were put into the system at a later time, it became necessary to use a second pump to increase the overall lifting rate of water from the lower to the upper tank, to match the increased draw-off from the upper tank. This extra pump delivered water directly to the upper tank without passing it through either the filter or ultraviolet sterilizer and although this obviously added un-filtered and un-sterilized water to the upper tank, the system was found to work successfully for up to 3 months without any apparent bacterial decomposition occuring in the water - providing that adequate care was taken to avoid overfeeding animals in the stock tanks. The sea water was checked periodically for chlorosity, pH and density but these were found to remain fairly constant over a period of 2 to 3 months, requiring little corrective attention. The whole aquarium room was thermostatically maintained at approximately 11°C but a separate freon-filled cooling coil, of stainless steel, was immersed in the water of the upper supply tank and this maintained the sea water temperature at 11 ± 1°C. The sea water in the finally modified two-tier system (Fig. A) was renewed every 2 to 3 months but over this period the system appeared to be successful in maintaining clear running sea water for the purposes of keeping live animals in readiness for experimentation.

Whilst the previously described modifications to the two-tier tank system were being considered, it was also decided that experimental tanks in the laboratory should be equipped with filtered and if possible running sea water. Initially, a filtering method without running sea water was used - air-powered aquarium filters containing acrylic wadding being placed in the tanks. In conjunction with this, ozone was bubbled into the sea water from a

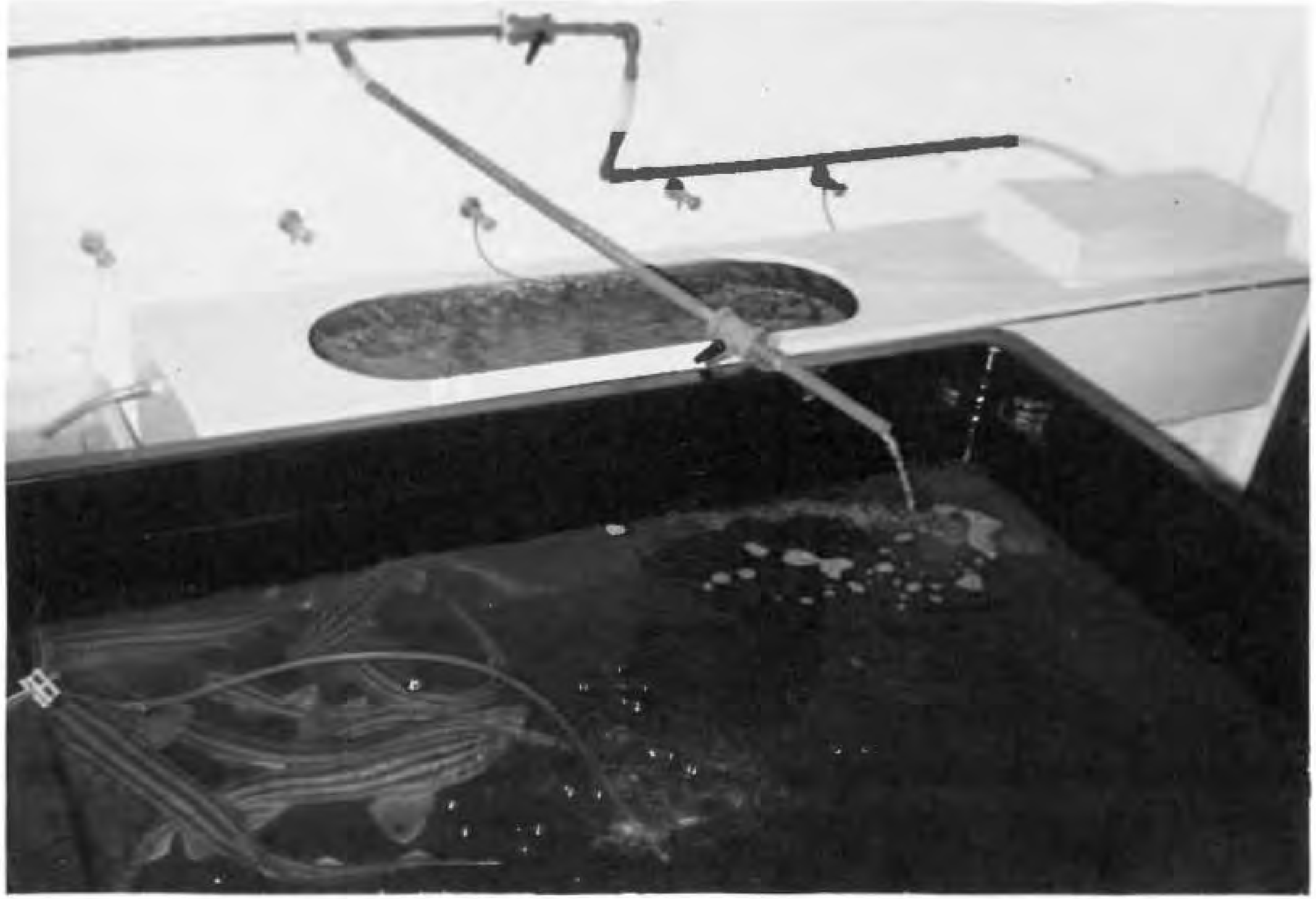

PLATE 1. View of the two stock tanks in Aquarium Room 2, looking down and towards the partition wall, from position X on Fig. A.

commercial discharge unit. This system worked for a short while but it became necessary to use considerable amounts of ozone after feeding fish, to neutralise the bacterial build up. At saturation concentration, ozone was found to be detrimental to dogfish, producing irritation of the gill epithelia, and the use of ozone as a sterilizing agent was discontinued. At this time, the two-tier system, supplying the stock tanks, had been in operation with diatomaceous earth as the filter medium but, as previously mentioned, this was found to require attention too often and it was decided to replace the earth with activated charcoal. At the same time, activated charcoal was added to the filters in the experimental tanks, with improved results, although without running sea water the system was still far from satisfactory. A prototype system using commercial pumps in conjunction with a filter containing acrylic wadding and activated charcoal and passing the water under ultraviolet fluorescent tubes, was then tried out on one of the experimental tanks. This proved wholly successful in providing sterilized filtered sea water to the experimental tank and the system, in principle, was extended to all experimental tanks immediately.

The final system used on all experimental tanks, and without which the present research would not have been possible, relied on the use of the compact German 'Eheim 386' and the smaller model '388' power filters. These filters use a constant-rating mains-powered centrifugal pump which draws sea water through a bag of activated charcoal and then polyester filter fibre. The outflow from the pump was, in each case, coupled to an ultraviolet sterilizer tube, which was specially designed for this system and consisted of a glass condenser tube with inlet and outlet couplings,

surrounding and sealing a mercury-vapour U.V. tube (Fig. B). The unit acts in a similar manner to a water cooling jacket around the central U.V. tube and ensures that sea water passing through the jacket receives high energy ultraviolet light, being in direct contact with the mercury-vapour tube. The flow rate through the jacket was adjusted to ensure that water passing through the jacket was in direct contact with the U.V. tube for about 8 to 10 seconds. At the intensity of radiation produced by the tube, and the proximity of the water to it, this was considered sufficient to ensure 100% kill of most micro-organisms in the sea water. Prototype jackets were made of Perspex but this was found to 'craze' after intense illumination for any length of time and later jackets were made of glass. Insufficient jackets were available for all tanks and on other tanks, sea water was passed along a trough just below the U.V. tube, in a similar system to that finally used on the two-tier system. This was found to be equally successful, providing the water was in close proximity to the tube for over 8 seconds.

This sytem was used successfully on experimental tanks for nearly 3 years and was found to require a minimum of cleaning and servicing - both of which are extremely easy with these filter units which can be quickly dismantled and cleaned out whenever necessary. In retrospect, it seems quite likely that the use of these units together with U.V. tubes, on the experimental tanks, has produced a more efficient filtering and sterilizing system than that which was operated on the large two-tier system supplying the stock tanks.

The sea water in the experimental tanks was regularly monitored for pH, salinity, osmolarity and temperature, according to the

PLATE 2. The main laboratory showing experimental tanks with power filters (foreground and right centre). A UV sterilizer unit can be seen above the foreground tank. Poor quality of picture due to reproduction of negative from colour slide.

experimental procedures in use at the time. The tendency for pH to drop slightly, with constant recycling, was counteracted by the addition of deionised water rendered slightly alkaline with Na_2HPO_4 buffer and NaOH (in the cases of medium dilution), and also by partial replacement of sea water, at regular intervals.

CHAPTER 4

HYPO-OSMOTIC REGULATION COUPLED WITH REDUCED METABOLIC UREA.*

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Hypo-osmotic Regulation Coupled with Reduced Metabolic Urea in the Dogfish *Poroderma africanum*: An Analysis of Serum Osmolarity, Chloride, and Urea

G. P. Haywood

Department of Zoology, University of Cape Town; Rondebosch, Cape Town, Republic of S. Africa

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G. P. Haywood

Department of Zoology, University of Cape Town; Rondebosch, Cape Town, Republic of S. Africa

Abstract

"Pyjama sharks" *(Poroderma africanum)* were exposed to a wide range of salinities, over which blood serum was analysed for osmolarity, chloride and urea concentrations. Fish were divided into two groups, those fed twice weekly (high intake), and those fed once a month (low intake). Both groups were exposed to the same salinity range. "High intake" fish showed the characteristic elasmobranch osmolarity picture, with serum values slightly hyper-osmotic at all times. "Low intake" fish, however, showed a degree of hypo-osmotic regulation. Serum values for both groups overlapped at very low salinities. Serum urea was also affected by diet, so that again two distinct sets of values were produced, again with overlap at the lower salinities. When previously well-fed fish were starved over a period of one month, serum urea and osmolarity decreased simultaneously. Consequently, it is felt that serum osmolarity is directly related to serum urea levels. Serum chloride was not found to be affected by diet, both groups showing the same change in blood values when exposed to the same change in salinity. It is shown, however, that a reduction in food intake, over a period of more than a fortnight, can reduce metabolic urea to the extent of depressing serum osmolarity and, hence, shift the ionic and osmotic equilibrium between the fish and the sea water. This may result in varying degrees of hypo-osmotic regulation.

Introduction

It is generally recognised that marine elasmobranchs, like their teleost counterparts, contain blood salts at about one-half sea-water concentration, so that serum-chloride values lie in the region of 250 mM/l (Chaisson, 1930; Smith, 1931; Burger and Hess, 1960; Burger, 1962; Price, 1967; Price and Creaser, 1967). Unlike teleosts, however, marine elasmobranchs have evolved the technique of reabsorbing and retaining urea in the tissues and body fluids (Smith, 1936; Kempton, 1953), so that serum osmolarity remains just greater than that of the external sea water. This greatly reduces their osmotic problem, so that they do not need to drink sea water (Smith, 1931) and excrete salts, as do teleosts (Keys and Willmer, 1932; Smith, 1932). However, in common with teleosts, they still have the problem of a natural and continuous diffusion of salts into the fish from the external sea water, where the concentration is higher. This is compensated for by salt excretion in the urine, and also by secretions of the rectal gland (Burger and Hess, 1960; Burger, 1962, 1965). It seems that there is also salt transfer at the gill epithelium (Maetz and Lahlou, 1966; Payan and Maetz, 1970, 1971).

When placed in diluted sea water, the osmolarity of an elasmobranch's blood falls and, conversely, when these fishes are exposed to concentrated sea water, the osmolarity rises (Scott, 1913, Chaisson; 1930; Margaria, 1931; Burger, 1965; Price and Creaser, 1967). This has been attributed largely to changes in concentration of serum urea (Smith, 1936; Price and Creaser, 1967; Goldstein *et al.*, 1968; Goldstein and Forster, 1971; Vlaming and Sage, 1973), but Burger (1965) found that previously starved spiny dogfish (*Squalus acanthias*), on being fed once more, showed no appreciable rise in tissue-urea concentrations, although serum osmolarity rose. A possible explanation for this is discussed below.

Whilst most workers have agreed on the effect of salinity changes upon the osmolarity of elasmobranch blood, the effect upon blood-salt levels seems less clear. Price and Creaser (1967) stated that, when exposed to a low salinity, the serum chloride level in the skate *Raja eglanteria* was depressed, although not greatly. Burger (1962, 1965), however, has indicated that, in the spiny dogfish *Squalus acanthias*, serum chloride is resistant to dilution of the medium, although he has shown that chloride can drop to about 220 mM/l. Recent work on the little skate *R. erinacea* (Goldstein and Forster, 1971), the sting-ray *Dasyatis sabina* (Vlaming and Sage, 1973) and the lemon shark *Negaprion brevirostris* (Goldstein *et al.*, 1968) support the present findings and those of Price and Creaser (1967).

It was decided to base this investigation of ionic regulation on chloride rather than sodium since, at the time of experimentation, the equipment and technique for sodium determination was not considered satisfactory. Also, serum shows higher values for sodium than for chloride in most elasmobranchs and, therefore, chloride can be used to give a minimal index of salt value.

Blood samples from *Poroderma africanum* over the whole salinity range were analysed for trimethylamine was 200 mM/l and 315 mM/l, respectively. In normal sea water (545 to 555 mM/l Cl), the serum had a chloride value of about 260 mM/l, which decreased to 220 mM/l when the external salinity was 375 mM/l Cl. Serum chloride rose to 315 mM/l when the fish were immersed in water of about 700 mM/l Cl. Diet had no effect upon serum chloride and, consequently, both groups were included in the above curve.

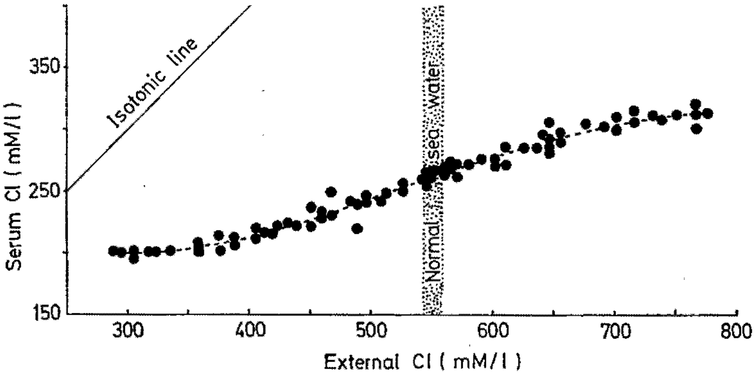

Fig. 1. *Poroderma africanum.* Variation in serum chloride with modification of external salinity

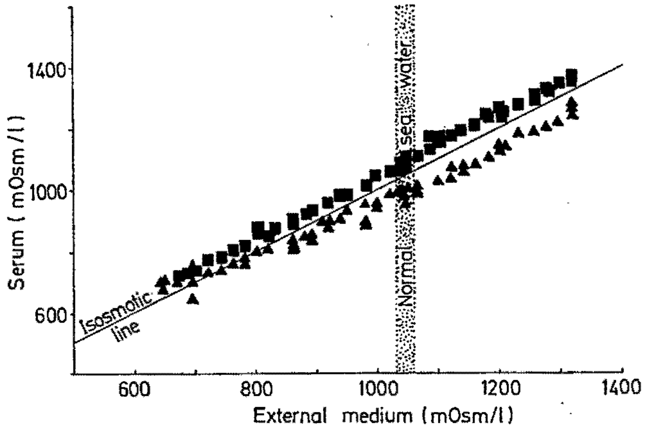

Fig. 2. *Poroderma africanum.* Combined effects of diet and salinity upon serum osmolarity. Squares: "high intake" fish; triangles: "low intake" fish

The results of variation in external medium upon serum osmolarity are shown in Fig. 2. A clear difference can be seen between "high intake" and "low intake" fish. At the lower tolerance limit of 650 mOsm/l, fish from both groups showed similar serum osmolarities, in the order of 700 mOsm/l but, as the external medium was raised towards 850 mOsm/l, the two groups showed significant differences (slope difference $P < 0.05$). Above an external osmolarity of 850 mOsm/l, the two sets of points were well separated, with a serum difference of 60 to 100 mOsm/l. In concentrated sea water (1060 mOsm/l upwards), this difference increased to 120—140 mOsm/l, and the regression coefficients for this part of the graph were shown to be significantly different ($P < 0.01$). The upper tolerance limit was 1320 mOsm/l.

A similar trend was shown by serum urea concentrations. Over a range of external media, from 700 to 1320 mOsm/l, blood values for the two groups were quite distinct (Fig. 3). Serum urea was shown to range from 215 to 550 mM/l, with "normal sea water" values of 455 to 465 mM/l and 405 to 415 mM/l for "high intake" and "low intake" fish, respectively. When replotted on a semi-logarithmic scale, a straight-line relationship was produced. This was similar to Fig. 2, and the initial slope difference was again found to be significantly different ($P < 0.001$). Whilst the semi-logarithmic plot did not show statistically significant differences in regression coefficients, it can nevertheless be seen that Fig. 3 shows a visible disperiods of up to 24 h in 87 to 95% sea water did not change plasma chloride, and fish which were kept in running diluted sea water for 3 to 9 days were able to stabilize plasma chloride, although it was found to drop to 220 mM/l in many cases. This latter fact seems in itself a contradiction of the earlier statement. It should also be remembered that the running sea water used was of fluctuating salinity "for the most part in the 72% to 82% range, with excursions up to 6 hours in 60% and 92%" (Burger, 1965). Consequently, the salinity at any given time appears to have been fluctuating and, since Burger has further stated that it took 48 h of dilution of the medium to affect fully the fish's internal composition, it is felt that the experimental parameters used by Burger were possibly not severe enough to show any low-salinity effects on the fish.

Price and Creaser (1967) showed that, for the clearnose skate *Raja eglanteria*, exposure to dilutions of medium produced depressions in serum chloride as well as urea concentration. When exposed to higher salinity, serum chloride was found to increase, although only in small amounts. Again 48 h was required for attainment of osmotic and ionic equilibrium with the environment, after a change of more than 2.4‰ or 40 mM/l Cl of the medium. Goldstein and Forster (1971) have shown that, in the little skate *R. erinacea*, transference gradually into half-strength sea water, from full-strength, resulted in reductions of plasma urea and chloride by 45 and 30%, respectively. Similar experiments with the lemon shark *Negaprion brevirostris* (Goldstein *et al.*, 1968) resulted in reduction of plasma urea and chloride by 55 and 20%, respectively. In the sting-ray *Dasyatis sabina*, Vlaming and Sage (1973) reported that "Plasma NaCl concentrations in *Dasyatis* are reduced to a greater extent by environmental dilution than are urea levels, until the external medium osmolarity falls below 350 mOsm per l".

Present results show that serum osmolarity and urea concentration vary with external salinity, in keeping with previous findings. It was shown by Scott (1913) that, when an elasmobranch is placed in diluted sea water, its osmotic pressure falls and, conversely, when in concentrated water, rises. However, all previous measurements of serum osmolarity show serum values slightly higher than the medium, and it has become generally accepted that serum is always maintained slightly hyper-osmotic to the sea water (Burger and Hess, 1960; Burger, 1965), in order to effect a slight endosmosis. As a result, marine elasmobranchs are not considered to drink the medium (Smith, 1931), as do marine teleosts.

The variation in serum urea and serum osmolarity seem closely linked with respect to the superimposed diet effect. According to Thorson *et al.* (1967), elasmobranch body fluids have shown urea nitrogen in the range of approximately 750 to 1300 mg/100 ml. If expressed in similar units, "high intake" fish showed urea nitrogen levels of 1260 to 1290 mg/100 ml, and fish on "low intake", about 1120 to 1150 mg/100 ml, in normal sea water. Whilst both sets of values lie within Thorson *et al.*'s figures, a difference of about 140 mg/100 ml is visible between the two groups. By comparison with the results of several workers presented in "Fish Physiology" (Hoar and Randall, 1969), *Poroderma africanum* appears to have serum urea levels a little higher than other species quoted (320 to 440 mM/l), there being a considerable amount of species variation.

Both urea and osmolarity show a tendency to stabilize at the lowest salinities. Watts and Watts (1966) have indicated that urea synthesis in elasmobranchs is stimulated by dilution of the medium, which might explain the slight "tail off" — although this should surely become apparent before such drastic dilutions are achieved. The effect of medium dilution upon urea production does not appear to be as noticeable for this species as the effect of diet.

So far little previous work has been done on actual feeding effects upon serum osmolarity and urea levels. Indeed, no previous thought appears to have been given to how elasmobranchs may fare under conditions of famine, and the consequential effects upon body fluids. From present findings, it is concluded that, when fed a regular and abundant meal, *Poroderma africanum* are able to maintain a high serum urea concentration and a correspondingly hyperosmotic serum (about 50 to 60 mOsm/l), at all salinities. However, with more infrequent feeding, the osmotic pressure of the serum falls below that of the medium. Furthermore, the link between diet and serum osmotic pressure is demonstrated in the experimental results presented in Fig. 4. This is to be expected, since serum urea results from metabolism of ingested proteins.

It has been shown, for *Poroderma africanum*, that the two serum osmolarity lines produced from the two diet groups represent fish at two extremities of a scale. Whilst it was found quite easy to keep fish alive without food for periods in excess of 2 months, adverse effects were noticed after about the sixth week and, consequently, fish being fed only once a month ("low intake") were deemed to be at "starvation" level (i.e. the absolute minimal feeding required to just maintain life, without producing abnormal body functions). From the results, as expressed in Fig. 4. it seems that, during the starvation period, there is a gradual decline in both serum urea and osmolarity until the two level off, due probably to metabolism of body protein. It would, therefore, also appear that for fish fed only once a month, serum values rise somewhat directly after feeding, but then decline again over the starvation interval. Since this effect was somewhat anticipated, blood samples were only taken just before each feed, as previously mentioned, in order

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Author's address: Mr. G. P. Haywood
Department of Zoology
University of Cape Town
Private Bag Rondebosch
Cape Town
Republic of S. Africa

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CHAPTER 5

THE EXCHANGEABLE IONIC SPACE, AND SALINITY EFFECTS UPON ION, WATER AND UREA TURNOVER RATES.*

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ABSTRACT

Using radio-isotopes, the turnover rates of sodium, chloride, water and urea were measured, at different salinities, in the Pyjama shark, Poroderma africanum (Gmelin). The exchangeable ionic space was also measured. Sodium turnover was found to be 0.24%/h, in normal sea water (467 mM Na/l, 550 mM Cl/l, 1020 mOsm/l.), and under similar conditions, chloride, water and urea showed turnover rates of 2.47%, 97%, and 0.08%/h respectively. Chloride and water turnover showed maximal values at this salinity. Exchangeable space was calculated, using Chloride-36, and was found to be 34.4% body volume or 32.4% ml/100g fresh weight. Present results show that chloride turnover is about ten times that of sodium. Water turnover rates are similar to those of other elasmobranch species, although urea turnover is somewhat lower. Salinity has a pronounced effect upon chloride and urea turnover rates.

INTRODUCTION

Much work has been presented concerning water and ion turnover in teleosts (reviewed by Maetz, 1970) but less has been done on the elasmobranchs. Maetz and Lahlou (1966) showed that the dogfishes, Scyliorhinus canicula and S. stellare had very low ionic turnover rates, chloride being exchanged at about ten times the rate of sodium. Burger and Tosteson (1966) have reported that the rate of sodium efflux in the dogfish Squalus acanthias is 0.5% body sodium/h. Chan et al. (1967) showed a sodium turnover of 0.74%/h, in the lip-shark, Hemiscyllium plagiosum.

Water turnover rates have recently been found as high as 157% and 167% total body water/h, in Scyliorhinus canicula and Raja montagu, although, in the ray Torpedo marmorata, the water flux was reported as 97%/h (Payan and Maetz, 1971).

Turnover of urea has been measured in Scyliorhinus canicula (Payan and Maetz, 1970) as 24 μM/100g/h and Goldstein and Forster (1971) have found similar rates, in the little ray Raja erinacea.

The flux measurements referred to above have all been conducted at one particular salinity. The present paper includes the effect of salinity-change upon these flux rates - a factor so far not examined by other workers in any detail. Previous measurements of exchangeable ionic space are few for elasmobranchs (Burger and Tosteson, 1966; Carrier and Evans, 1972), although the partitioning of body water and fluid compartments in groups of fishes, including Chondrichthyes, has been studied (Thorson, 1958, 1961; Holmes and Donaldson, 1969). Techniques similar to the present one have been employed to determine the exchangeable sodium space (Mayer and Nibelle, 1969) and chloride space (Kirsch, 1972) in fresh-water and sea-water eels.

MATERIALS AND METHODS

Pyjama sharks (Poroderma africanum Gmelin) were collected and kept in the laboratory as described previously (Haywood, 1973). Fish ranged between 0.5 and 0.8 m in length and 1 and 3 kg in weight. Both sexes were represented approximately equally. In the laboratory, animals were kept at 13°C and fed teleost meat and small whole cephalopods at regular intervals, until 48 h prior to experimentation. It was found impractical to feed animals any closer than this to the commencement of experiments, due to regurgitation after anaesthetic.

TURNOVER RATES

For efflux measurements, animals were injected intravenously with known quantities of radio-isotopes, in isotonic saline, and immediately placed in a small volume of sea water until superficial bleeding ceased. Fish were then placed in the experimental tanks for 6 to 10 h, to allow complete mixing of radio-isotope and body fluids. Blood samples were then taken over regular time-intervals and serum expressed from the blood (after Haywood, 1973) to determine serum radioactivity. Experiments were done at different salinities for each isotope used - with the exception of Sodium-22, which was used in normal sea water only. The reasons for this are discussed later. All modifications to the external salinity were made slowly, as previously described (Haywood, 1973) and fish were allowed a minimum period of 60 to 72 h acclimation time in the new medium prior to experimentation.

The above technique of radio-isotope administration was not feasible for tritiated water (^3H_2O), due to its high efflux rate. Instead, fish were placed in a loading solution (high radioactivity) of the isotope for 6 to 10 h. In all cases, the sea water volume in experimental tanks was made large enough to reduce radioactive back-flux to negligible proportions.

The radioactive efflux was calculated from counts of successive blood samples, using the formula;

$$K = \frac{1}{T} \ln \frac{Ao}{At}$$

where K is the rate constant of turnover (fraction of the exchangeable substance per hour), T is the length of experimental period in hours, Ao blood radioactivity at time 0 and At the blood radioactivity at time T.

EXCHANGEABLE IONIC SPACE

The volume of the distribution space (E) of an ion may be measured by dilution of a radio-isotope in the internal medium (Maetz, 1956; Mayer and Nibelle, 1969; Kirsch, 1972). Once equilibrium is achieved, the following equation is true (after Kirsch, 1972):

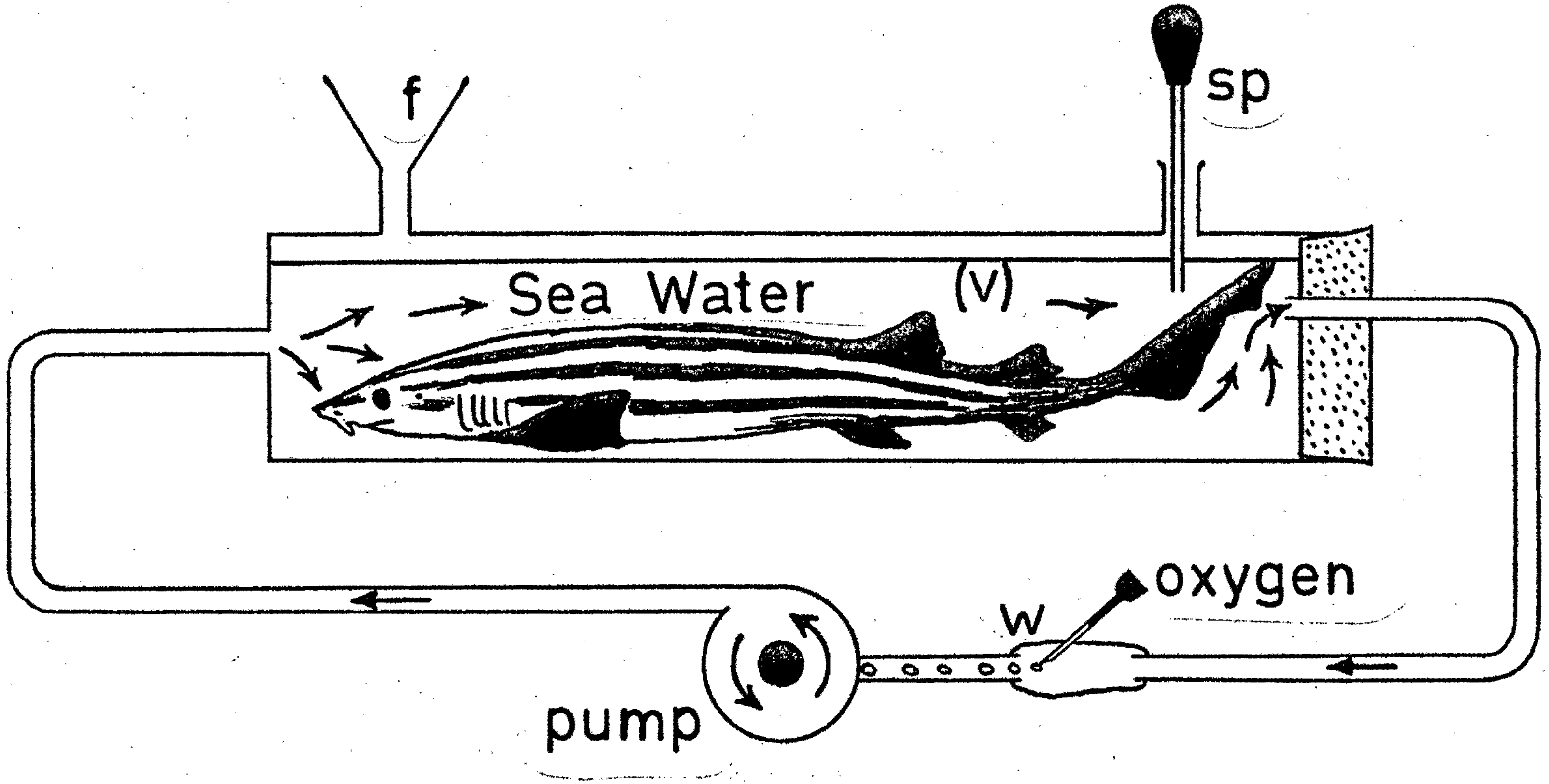
$$E = \frac{Qo - dQt}{Ct}$$

Qo is the quantity of tracer injected into the fish at time 0, dQt represents tracer lost to the external medium at time T and Ct is the blood concentration of radio-isotope at time T.

The above equation was originally employed to determine the exchangeable chloride and urea spaces in <u>Poroderma</u> <u>africanum</u> but

FIGURE 5. CHLORIDE-SPACE APPARATUS

Apparatus is filled with a measured volume (v) of sea water via funnel (f), after fish is secured in Plexiglass tube (px). Water samples are withdrawn by sampling pipette (sp) and oxygen is bubbled into the system through a needle embedded in a plastic 'window' to observe bubbling rate.



even though the specially designed apparatus (Fig. 5) made the calculation of dQt relatively easy, slight superficial bleeding immediately after injection of tracer made it virtually impossible to assess Qo to the degree of accuracy desired. Consequently the following technique, which obviates the need to know Qo, was devised and used instead.

After injection of tracer and attainment of equilibrium, the fish was exposed to a known volume of sea water. At time 0, a blood sample was taken from the fish and at the same time, 10 ml of the sea water surrounding the fish were also withdrawn - both for radioactive assay. Ten millilitres of fresh sea water were then added back to the system, to maintain the original sea water volume. After an experimental period (T), the procedure was repeated.

If Co = blood concentration of tracer at time 0

Ct = blood concentration of tracer at time T

Wo = tracer concentration in sea water at time 0

Wt = tracer concentration in sea water at time T

V = volume of sea water

then, since the system is closed, with respect to tracer, the loss of tracer from fish must be equal to the gain in tracer by sea water.

i.e. $(Co - Ct) E = (Wt - Wo) V$

Hence the distribution space (E) of the fish is given by

$$E = \frac{(Wt - Wo)\ V}{(Co - Ct)}$$ (All blood and water counts per unit volume)

Each fish was injected with radio-isotope, as described, and allowed to attain equilibrium overnight. The fish was then

PLATE 3. Chloride-space apparatus as seen from the opening end, showing 'O'-ringed door and 4-bolted pressure plate. The Eheim 386 pump is shown on the right and the oxygen line (above apparatus) supplies hypodermic needle in sea water return tube (adjacent to upper right pressure plate bolt).

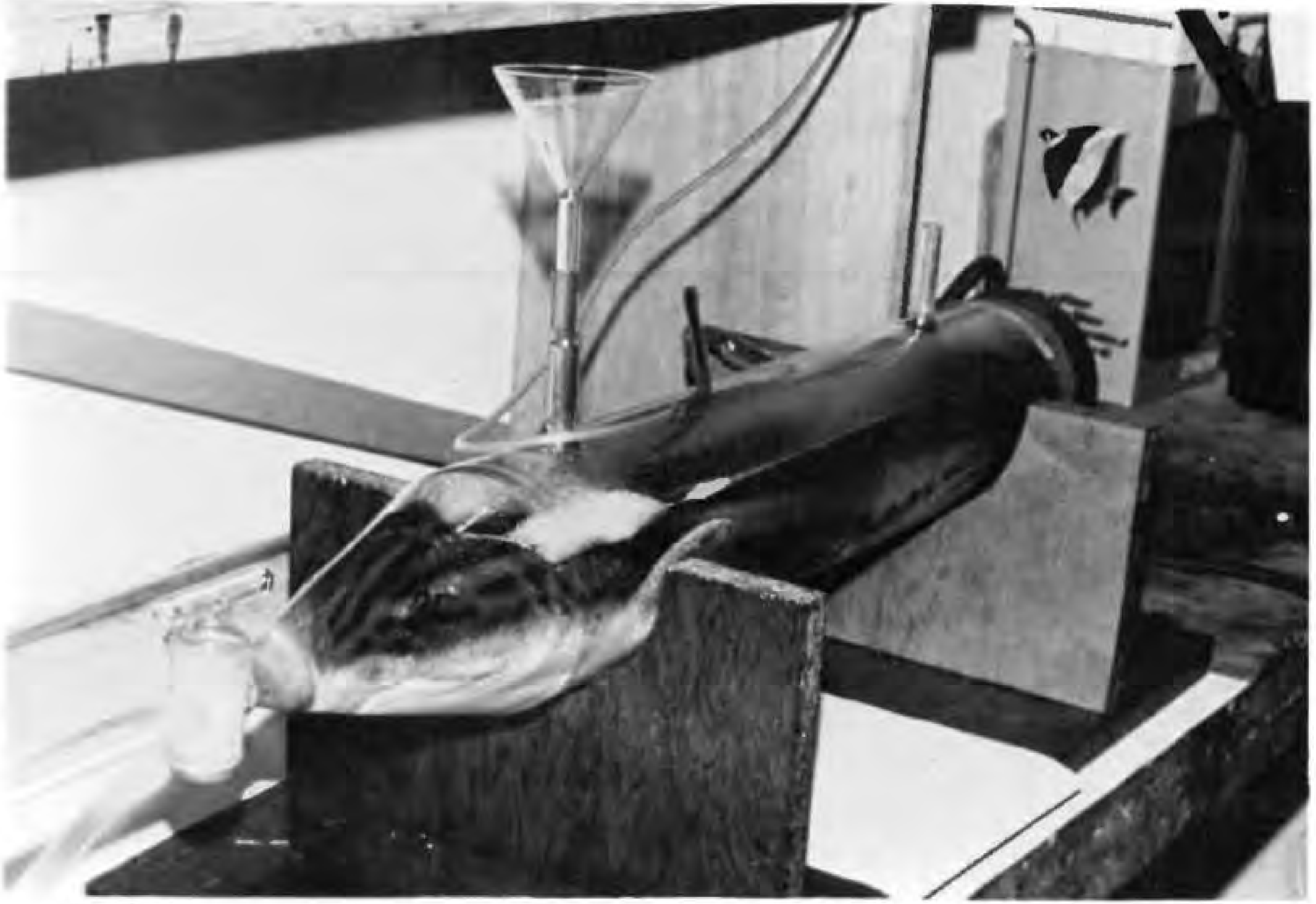


PLATE 4. Chloride-space apparatus as seen from sea water inflow side, showing animal in position with sea water flowing into the mouth and through the gills. Slight froth above gills is due to excessive oxygenation of inflowing medium. Oxygen line is visible around funnel stem.

anaesthetised in sea water containing 0.03% MS-222 (tricainemethanesulphonate), weighed and its displacement volume measured. A blood sample was then taken and the fish immediately placed in the Plexiglass tube of the specially designed apparatus (Fig. 5). A known volume of sea water (2.5 to 3.0 l) was then added and, after a few minutes of recycling by the pump to ensure full mixing, 10 ml was drawn off for assay and the volume replaced as described above. At the end of the experimental period (10 to 12 h), a further 10 ml water sample was removed from the tube and the fish immediately removed for a blood sample.

Throughout the experimental period, the fish was kept inactive by the addition of a small amount of MS-222 to the recycling water. Oxygen was diffused into the water via the pump (Fig. 5) to reduce the degree of anaesthesia as required.

This apparatus was found to be impractical when measuring urea space, in view of the very long time period (10 days) required for sufficient radio-isotope to be lost from the fish. The fish were therefore placed in small polythene tanks containing 10 l of sea water, mixed and cleaned by small air-powered filters. The same experimental procedure was adopted.

BLOOD MEASUREMENTS

After separation from whole blood by centrifugation, serum for liquid-scintillation counting was mixed with an equal volume of 5% trichloroacetic acid, allowed to stand overnight at 5°C, and recentrifuged. This procedure prevents the "colloidal effect" (due to suspended proteins) sometimes produced when adding serum to scintillation fluid. Beckman TLA "Fluoralloy" /Triton X-100 (toluene-based) scintillation mixture used for counting Chloride-36 and ^{14}C-urea but tritiated water was counted using Schwarz-Mann "Unogel".

Sodium-22 activity was measured using a Packard Model 578 Gamma Scintillation Spectrometer, serum samples being diluted in isotonic saline. Chloride-36, ^{14}C-urea and tritiated water were measured using a Packard Tricarb Model 3380 Liquid Scintillation Spectrometer. Quench corrections were made using the Automatic External Standardization system.

Serum chloride concentration was measured after Schales and Schales (1941) and urea after Pré _et al_. (1968). A Knauer platinum-thermistor osmometer was used to measure serum osmolarity. Serum sodium was determined by flame emission spectroscopy, using a Unicam SP 1900 Atomic Absorption Spectrophotometer.

RESULTS

TURNOVER RATES

Turnover rates for fish in normal sea water are presented in Table 1. With the exception of sodium, these values are taken from Figs. 6 to 8, representing turnover rates at different salinities. The latter are also expressed numerically in Tables 2 and 3. Turnover rate is expressed as a percentage of the exchangeable substance in the body exchanged per hour and is obtained by multiplying the flux constant (K) by 100. No appreciable difference was found between the two sexes, and results are therefore presented irrespective of sex. Each fish was used only once at each salinity.

Fig. 6 indicates the effect of salinity change upon chloride turnover. It can be seen that turnover is maximal in normal sea water. Fig. 7 presents the effect of salinity change upon urea turnover. Urea turnover appears fairly constant in the mid-salinity range but is affected at the extremes. The effect of salinity change upon water turnover is presented in Fig. 8. Here, salinity effect is far less marked but again turnover is maximal in normal sea water.

EXCHANGEABLE IONIC SPACE

Measurements were made of both exchangeable urea space and exchangeable chloride space. Since however, the measurements of urea space gave inconsistent results, the estimation of the volume of exchangeable ionic space has been based purely upon the chloride space results. Exchangeable chloride space can be expressed either as a percentage of the total displacement volume of the fish, or as volume per 100g fresh weight. Based on a calculated density, for Poroderma africanum, of 1063 g/l, the exchangeable chloride space was found to be 34.4 ± 8.6 % of the total volume, or 32.4 ± 8.0 ml/100 g (n = 16).

Table 1. Serum concentrations and turnover rates of the substances examined, for <u>Poroderma africanum</u> in normal sea water.

Substance	Serum concentration (Mean ± S.D.)	Turnover rate (Mean ± S.D.)
Na	238 ± 7 mM/1 (n = 10)	0.24%/h ± 0.01 (n = 8)
Cl	261 ± 5 mM/1 (n = 10)	2.47%/h ± 0.09 (n = 8)
H_2O	———	97%/h ± 4 (n = 7)
Urea	462 ± 13 mM/1 (n = 6)	0.08%/h ± 0.01 (n = 7)

Table 2. Chloride and water turnover rates for <u>Poroderma africanum</u> in salinities other than normal sea water.

		External medium mMCl/l			
		450	500	600	650
Turnover rate (Mean ± S.D.)	Cl^-	1.15%/h (n = 2)	1.89%/h ± 0.23 (n = 5)	1.38%/h ± 0.14 (n = 4)	1.80%/h ± 0.25 (n = 3)
	H_2O	83%/h ± 3 (n = 5)	81%/h ± 4 (n = 4)	86%/h ± 4 (n = 5)	85%/h ± 5 (n = 5)

Table 3. Urea turnover rates for *Poroderma africanum* in salinities other than normal sea water.

	External medium mOsm/l			
	750	900	1000	1170
Turnover rate	0.06%/h ± 0.01	0.08%/h ± 0.01	0.07%/h ± 0.02	0.11%/h ± 0.02
(Mean ± S.D.)	(n = 4)	(n = 5)	(n = 5)	(n = 5)

DISCUSSION

The measured sodium turnover rate of 0.24%/h, for Poroderma africanum in normal sea water, is rather low, by comparison with the findings of other workers. It is about half the value obtained by Burger and Tosteson (1966) for the spurdog, Squalus acanthias, and is well below the figure of 0.74%/h, reported by Chan et al. (1967) for the lip-shark, Hemiscyllium plagiosum.

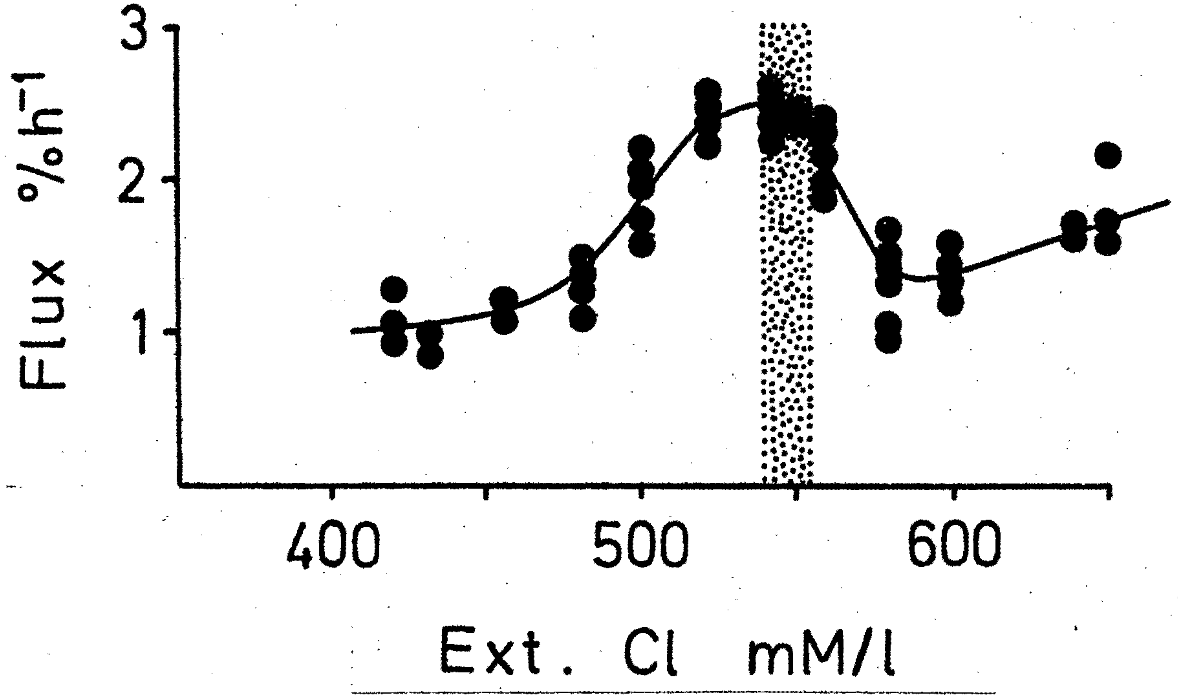
In view of the short time period (48 h), over which the investigation was carried out by Burger and Tosteson - without apparently allowing for the attainment of equilibrium between tracer and fish - no real comparison can be made between their efflux results and those presented here. It has been found, for P. africanum, that immediately after injection of radio-isotope, the apparent flux rate (as measured) is higher than the same rate, when measured again after about 6 h. This is attributed to inadequate mixing between tracer and body fluids in this short time period, and has been substantiated by Comar (1955). After mixing is complete (6 h), the measured efflux rate is invariably constant. Disregard for this mixing time could well result in a higher measured turnover rate than the true value. Moreover, the results presented by Burger and Tosteson (1966) appear to be based on measurements of only two fish. Chan et al. (1967) appear to have taken the mixing time into account and their turnover rate of 0.74%/h, for sodium in the lip-shark may be taken as a sound comparison. Unfortunately, no figures are available for chloride turnover in this species. Carrier and Evans (1972) have reported a sodium turnover rate of 0.46%/h ± 0.16, for the nurse shark, Ginglymostoma cirratum. Their measurements were carried out at 24°C, which is somewhat higher than the temperature at which most of the other quoted workers measured turnover rates (mostly between 12°C and 18°C). More recently (Carrier and Evans, 1973), they have reported a sodium turnover rate of 0.28%/h $\pm$ 0.16, in the fresh-water elasmobranch Potamotrygon - also measured at 24°C.

In view of inadequate handling and screening facilities, the prolonged laboratory use of a long-life gamma-emitter, such as Sodium-22, was considered unwise and consequently it was decided to experiment on 8 fish in normal sea water only. No salinity-effect studies were therefore carried out for sodium trunover.

The rate of chloride turnover, in P. africanum, was found to be ten times that of sodium. Maetz and Lahlou (1966) showed similar results in the two dogfishes Scyliorhinus canicula and S. stellare. Since P. africanum can be regarded as a Scyliorhinid-type (from physical appearance), the similarity is interesting. The ten-fold difference is not apparent in all species of elasmobranch; Carrier and Evans (1972) reported the difference as being only about four fold in the nurse shark (1.52%/h, for chloride turnover) and even less than this in the fresh-water Potamotrygon (0.22%/h, for chloride turnover).

The effect of salinity upon chloride turnover in P. africanum (Fig. 6) is quite pronounced. Turnover was maximal in normal sea water but high turnover was found even with slight dilution of the medium (down to 500 mM/l Cl). At lower salinities, the flux rate dropped towards 1%/h. The sharp peak in turnover rate at about normal sea water appears to be superimposed upon an otherwise gradual increase, with salinity increase. This could result from

FIGURE 6. Chloride turnover rate expressed against external chloride concentration. Stippled area denotes normal sea water range.



an increased inward diffusion of chloride at higher salinities being balanced by increased output. It has been shown (Haywood, 1973) that serum chloride only rises slowly with increase of external salinity. The overall curve of Fig. 6 indicates considerable reduction in body permeability to chloride, when fish are in unusual salinities (the species is considered to be stenohaline).

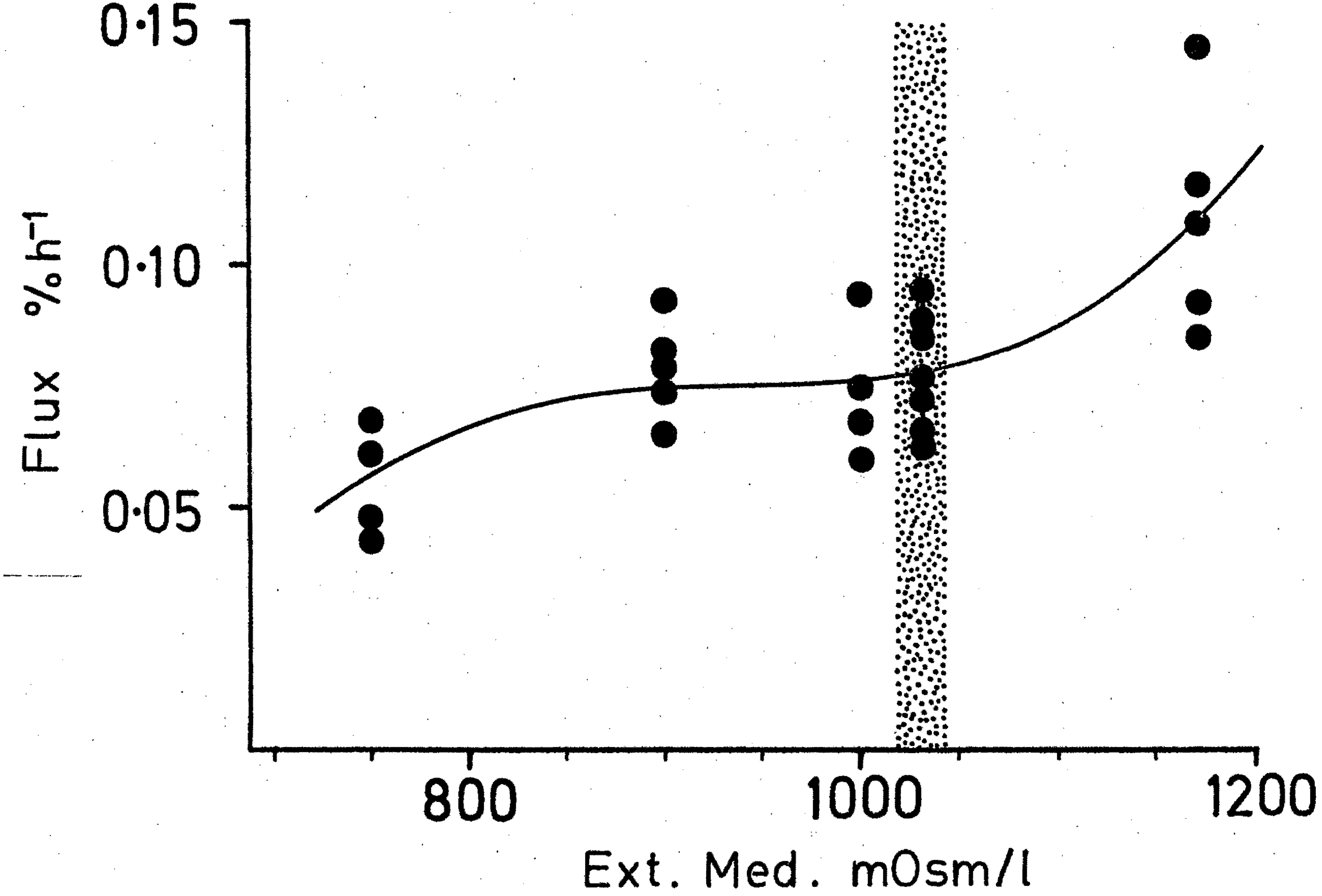
The present estimation of chloride space gives a volume greater than that of Thorson (1961), which was 28.3% body water - if the volume is assumed to be total extracellular fluid (blood plus interstitial fluid). Kirsch (1972) reported a chloride space of between 22.3 and 29.9 ml/100g for fresh-water and seawater - acclimated eels, although he reported that transfer from one salinity to another caused slight changes in chloride space. All present experiments were carried out over the mid-range of salinities, over which chloride space was assumed to be a constant volume. There is scope for future investigations into salinity effects upon exchangeable ionic space volume. Burger and Tosteson (1966) reported sodium space in the spurdog, Squalus acanthias, as below 25 ml/100g.

By taking chloride space into account, as exchangeable ionic space, the turnover of sodium and chloride can be expressed in absolute units, i.e. 18.5 µM Na/100g/h and 208µM Cl/100g/h, for P. africanum in normal sea water. Similarly, application of exchangeable chloride space to urea turnover, results in a calculated absolute figure of 12 µM/100g/h. This is half the value given by Goldstein and Forster (1971), for the little skate Raja erinacea, and also that for Scyliorhinus canicula as observed by Payan and

Maetz (1971). Since exchangeable ionic space seems no smaller than for other species, with serum urea at 462 mM/l, the very small urea loss in P. africanum must be attributed to the extremely slow turnover rate. Carrier and Evans (1972) have also calculated urea turnover in absolute units, as more than double the present findings - but their figures are based on two estimations of exchangeable urea space - 38 ml/100g and 69 ml/100g. All elasmobranchs so far studied have shown very slow urea turnover, which is not surprising in view of their specialized ability to reabsorb and retain urea in the tissues (Kempton, 1953; Boylan, 1967). Indeed this may be possible by the production of a low-urea environment in the vicinity of the terminal segments of the renal tubules, resulting in passive urea reabsorption (Boylan, 1972). Since Goldstein (1967) has determined the rate of urea synthesis in Squalus acanthias as approximately 27 μM/100g/h, it appears that in the elasmobranchs studied so far, urea synthesis always equals the diffusional loss across the epithelium. Urea synthesis would not be required at this rate for P. africanum, in view of the small loss and obvious high degree of impermeability to urea at the epithelium.

Salinity effect upon urea turnover, in the above species, is quite marked. Over the mid-range of salinities, turnover is relatively unaltered (0.08%/h) but in extremely low and high salinities, the rate decreases to 0.05% and increases to 0.1%/h respectively. The overall curve of Fig. 7 may be interpreted as higher body urea concentration causing increased passibe outward diffusion, at higher salinities. Reduction in external salinity will also lead to a fall in internal osmolarity, by adjustment of the serum urea concentration. This will lead to a decrease in the

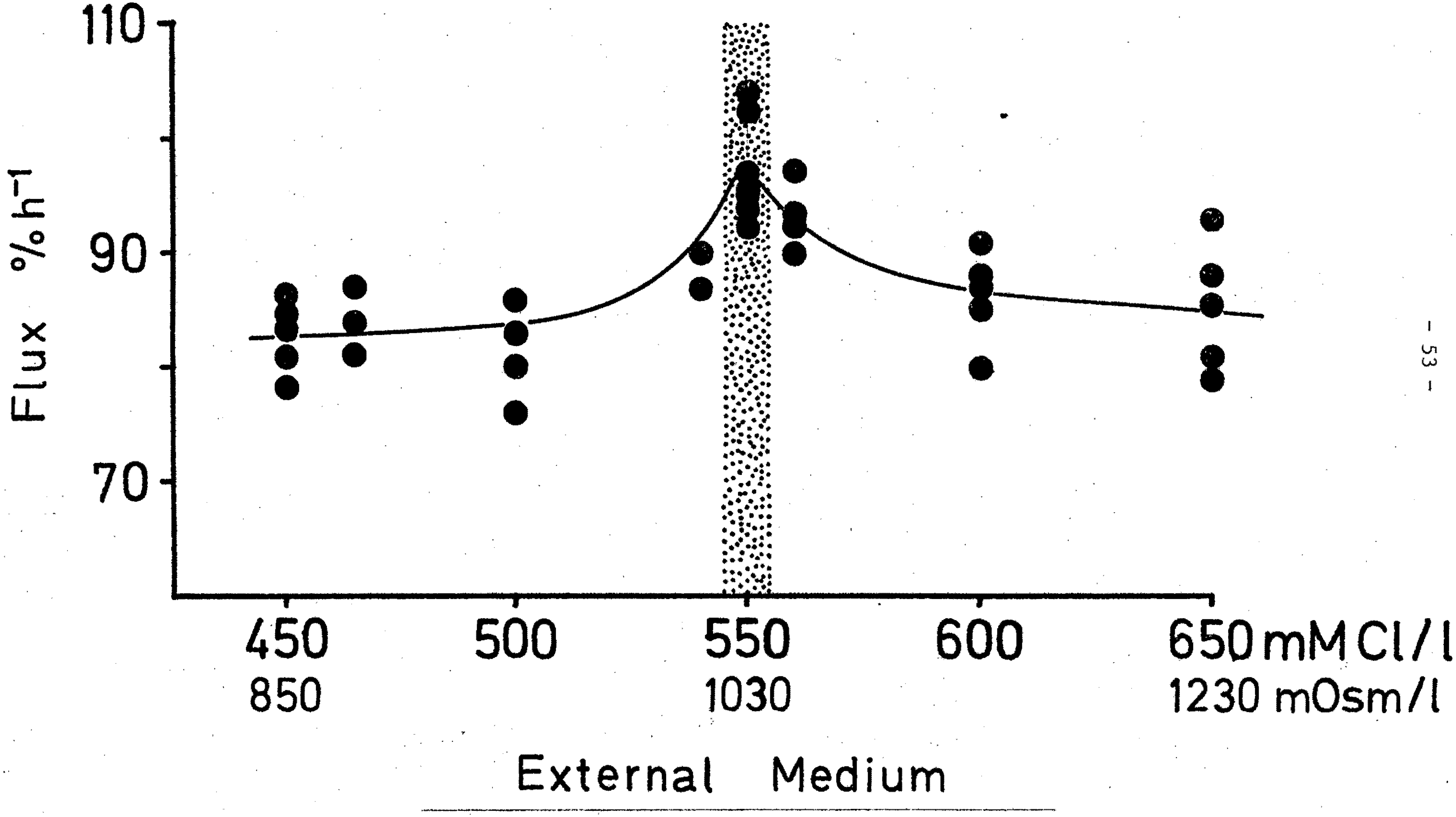
FIGURE 7. Urea turnover rate expressed against external osmolarity. Stippled area denotes normal sea water range.



urea concentration gradient between the body fluids and external medium, resulting in a parallel fall in urea turnover. Payan et al. (1973) found that, in skates gradually adapted to 50% sea water, branchial urea excretion decreased from 57 μmoles/100g/h to 20, in direct relation to the reduction in plasma urea concentrations from 589 mM/l to 227. There appeared to be no change in the permeability of skate gills to urea during adaptation. It is noteworthy that they mentioned these findings, regarding unchanged permeability to urea, as being contrary to certain other elasmobranchs. The extremes of the curve (Fig. 7) of present results suggest a drop in the otherwise high impermeability to urea, presumably resulting from the added stress of the drastically changed external medium.

Water turnover in P. africanum is extremely high, in keeping with findings for other elasmobranchs. This suggests a high degree of water permeability which argues against earlier suggestions (Price, 1967; Price and Creaser, 1967) that water permeability in elasmobranchs is low. Their suggestions seem to be based on a 48 h period being required for the attainment of osmotic equilibrium, after transfer to diluted medium. Since, however, similar findings have been reported for P. africanum (Haywood, 1973), it may well be that the equilibrium time is more dependant upon the slower ion fluxes, so that water permeability is not a limiting factor. Carrier and Evans (1972) suggested that water turnover might be limited by cardia output. Randall (1970), however, has reported a cardiac output of the order 150 ml/100g/h, for elasmobranchs. Assuming the total body water of the species to be 75 ml/100g fresh weight, as indicated by Holmes and Donaldson (1969), and assuming

FIGURE 8. Water turnover rate expressed against external osmolarity and chloride concentration. Stippled area denotes normal sea water range.



this water exchanges as a single compartment, this gives *P. africanum* a water turnover of 73 ml/100g/h - which could well be handled by cardiac output. Results of unpublished work indicate that much of this water turnover, and indeed ionic transfer, occurs at the gills. Maetz and Lahlou (1966) indicated that branchial fluxes were responsible for a large part of the overall fluxes and recent work by Payan and Maetz (1973) has shown definite branchial sodium transport mechanisms.

Salinity effect is much less pronounced on water turnover, than on ion turnover, but it appears that for *P. africanum* water turnover is maximal in normal sea water, decreasing towards 80%/h with dilution or concentration of the medium. Payan and Maetz (1971) have stated similar findings for *Scyliorhinus canicula*, *Raja montagu* and *Torpedo marmorata*, where transfer to 85% sea water resulted in decrease in water flux rates. Present results indicate only relatively small change in water flux with salinity change, which might be partly accounted for by only small changes in the osmotic gradient between blood and external medium as salinity is altered (Haywood, 1973), but may also be produced by a drop in body permeability to water, when animals are in unusual salinities. It has been shown (de Vlaming and Sage, 1973) that when stingrays are transferred to 35% sea water, from normal sea water, plasma osmolarity decreases by only 23%, and they suggested that the animals were more efficient (i.e. possibly less permeable) than other species studied. They further showed that stingrays maintain body water balance in diluted media. Measurement of water turnover in stingrays in dilute media would form an interesting comparison with present findings. It is interesting that both stingrays (de Vlaming and Sage, 1973) and clearnose skates (Price, 1967; Price and Creaser, 1967) show a greater osmotic gradient between blood and external medium, than does *P. africanum*, as salinity falls, so that in very dilute media the blood osmolarity of those two species is well above the isosmotic line.

In conclusion, *Poroderma africanum* shows certain physiological adaptations towards the stenohaline conditions of its environment, an important one of which is the reduction in body permeability to ions and water, when salinity conditions alter away from the normal. Nevertheless, present results, together with previous findings (Haywood, 1973), although carried out under laboratory conditions, in either direction, without any apparent adverse effects. It is therefore suggested that whatever factor prevents the species from penetrating brackish or hyper-saline waters under natural conditions, it is not an osmo-regulatory one.

CHAPTER 6

SODIUM, CHLORIDE AND WATER EXCHANGE ACROSS THE GILLS.*

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ABSTRACT

Under laboratory conditions the gills of the stiped dogfish (Poroderma africanum) were studied as a possible site of ion and water transfer between internal and external media. Haemotocrits showed that a drop in the external osmolarity produced increase in blood volume, measured as a decrease in blood p.c.v.'s (packed cell volume). Similarly, when fish were exposed to increased external osmolarity, the converse occurred, with resultant rise in p.c.v.'s. The use of phenol red showed that normally-fed fish did not drink the medium and hence dilution of the blood was most probably due to water influx at the gills. Hypo-osmotic fish (due to under-feeding) were found to drink the medium in appreciable quantities and dilution and concentration of external medium had a more pronounced effect on blood p.c.v.'s in these animals. The p.c.v.'s of normally-fed animals returned to initial values within 7 days after transfer to new medium, in most cases, but hypo-osmotic animals took longer to adjust to the new state of water balance. Surgical closure of rectal gland and urinary systems produced initial rises in serum sodium and chloride levels, but these reached equilibrium after 5 to 7 days, indicating compensatory regulation by some other organ, such as the gills. After removal of the sutures to the urinary systems of 3 fish, there was a noticeable drop in sodium and chloride levels of the serum in these individuals. All fish were kept for 14 days in the laboratory, with little change in blood composition (as measured) and with only the gills as regulatory organs in two of them. By using the radio-isotopes Chloride-36 and Sodium-22, it was shown that both ions are lost at the gills, against the concen-

tration gradient. Histochemical examination of gill tissue from several fish indicated that many cells contain high concentrations of chloride and are probably the site of chloride excretion. The number of such cells increased with increase in external salinity, and they were also abundant in tissue from hypo-osmotic animals and those with inoperative urinary and rectal gland systems. From these findings, it was therefore concluded that the gills have a definite role in the ion and water balance of this species.

INTRODUCTION

In 1930, Homer W. Smith formulated the hypothesis that marine teleosts drink large quantities of the external medium, from which water and salts are absorbed by the gut and salt is excreted at the gills, which therefore form an important osmoregulatory organ. Later work (Smith, 1931) led to the suggestion that such was not the case with marine elasmobranchs, as they were not found to drink the medium "as a rule". Under normal conditions, elasmobranchs have been shown to maintain slightly hyper-osmotic blood, by the reabsorption and retention of urea (Smith, 1931; Kempton, 1953) - thus making up the osmotic deficit between body fluids and external medium, which would otherwise result from the low blood salt level. Boylan (1967) showed that 95% of filtered urea is reabsorbed by the elasmobranch renal tubules compared with only 35% thio-urea - arguing for a possible active reabsorption mechanism. Later work by Boylan (1972) has however, suggested that, due to a tubular "low-urea environment", passive urea absorption may well occur in the elasmobranch kidney. Schmidt-Nielsen _et_ _al_.(1972) have suggested that sodium-linked urea transport occurs in the renal tubules of _Squalus_ _acanthias_, so that the actual mechanisms of urea reabsorption in elasmobranchs are still only partially understood.

The hypothesis that marine teleosts actively excrete salts at the gills was further strengthened by histochemical studies of gill tissue from several species. Keys and Willmer (1932) showed the presence of definite "chloride-secreting cells" in the gill epithelium of the eel _Anguilla_ _anguilla_, and later, similar cells were found in the gills of the euryhaline teleost _Fundulus_ _heteroclitus_ (Copeland, 1948, 1950) and also in gill tissue of fresh-water teleosts (Datta Munshi, 1964). Keys and Willmer were unable to find such cells in the gill tissue of *Scyliorhinus* sp. - the only elasmobranch examined by them - and they concluded that such cells are absent from elasmobranch gill tissue, so that the gills are not important as osmoregulatory organs. There were several objections to the ion-secreting idea of definite "chloride cells", as suggested by Keys and Willmer. Bevelander (1936, 1946) and Parry *et al*. (1959) objected on the grounds that these were not "special cells" but normal epithelial cells that were excreting the chloride. Doyle and Gorecki (1961), with one of the first ultrastructural investigations (all previous studies had been at the light microscope level only), found similar cells in elasmobranch gill tissue and concluded that such cells served other purposes as well as chloride secretion. Subsequent workers have reported "chloride cells" in several fish species and more recently definite ion-selective transport mechanisms have been described, in connection with these "chloride cells" (Maetz and Garcia Romeu, 1964; Maetz, 1969, 1971, 1972; Kamiya, 1972; Evans *et al*. 1973). As a result, the teleost gill is now regarded as an important osmoregulatory organ.

The suggestion that elasmobranchs do not excrete salts at the gills was further strengthened in 1960 by the work of Burger and Hess on the rectal gland, indicating its function as a salt gland. The hypothesis was put forward that the rectal gland was able to excrete a concentrated sodium chloride solution and in so doing, maintain ionic and osmotic balance in the elasmobranchs.

Later work with Sodium-22 radio-isotope (Burger and Tosteson, 1966) showed that sodium ions are transported between body fluids and external medium in the head region of the spiny dogfish *Squalus acanthias* - most probably at the gills. Maetz and Lahlou (1966) also showed that branchial efflux was responsible for a large portion of the total ionic efflux in *Scyliorhinus* spp. and Payan and Maetz (1971, 1973) have shown similar findings for the dogfish *Scyliorhinus canicula*. They have also reported the presence of "chloride cells" in the gills of the same species.

In the light of the above, it was decided to investigate the possibility of branchial ionic and osmotic regulation in *Poroderma africanum*.

MATERIALS AND METHODS

Pyjama sharks Poroderma africanum (Gmelin) were collected and kept in the laboratory as described previously (Haywood, 1975). Fish ranged in size between 0.5 and 0.8 m in length and 1 and 3 kg in weight. Both sexes were represented approximately equally. In the laboratory, animals were kept at 13 ± 1°C and fed teleost meat and small whole cephalopods at regular intervals until 48 h prior to the commencement of experimentation, as explained previously (Haywood, 1974a).

DRINKING OF THE MEDIUM

The indicator dye phenol red has been previously used (Smith, 1930) to demonstrate that marine teleosts drink the medium. Phenol red does not enter the blood capillaries to any appreciable extent and can only appear in the alimentary canal as result of direct ingestion of the external medium. Furthermore, the fact that the dye is not readily absorbed from the intestine leads to its extensive concentration in the gut, as water and salts are absorbed.

Six fish, which had been kept unfed for over 21 days, and which exhibited hypo-osmotic serum, were placed in sea water of similar salinity to the acclimation medium (slightly below normal sea water) but containing sufficient phenol red to colour it deep pink. After 24 h, the fish were removed, lightly anaesthetised in sea water containing MS-222 (tricainemethanesulphonate) and stomach fluid withdrawn by means of a thin flexible tube with attached syringe, passed down the pharynx. Fluid from the posterior gut was sampled, where possible, by a similar arrangement introduced into the rectum. The presence of dye in the stomach fluid was made immediately apparent (if present) by the addition of 1 pellet of NaOH to the sample, increasing the pH and rendering any phenol red present dark red in colour. Three control animals exhibiting hyper-osmotic serum, were also treated as above.

Other animals were also treated with phenol red, as an investigation into possible drinking of the medium, but this was done in connection with other experiments and is briefly described, where appropriate, in the following sections.

HAEMATOCRITS

Animals were acclimated to sea water of known osmolarity (see results) for a 1-week period, after which, a blood sample was taken from each fish (after Haywood, 1973). Serum osmolarity was measured and haematocrits (expressed as packed cell volumes or p.c.v.'s and measured as a percentage of total sample volume) were read, after centrifuging the blood in heparinized tubes at 12,500 r.p.m. (16,000g) for 6 minutes. Eleven animals, in two groups (6,5) were then transferred to diluted sea water (for actual values see Tables 4 and 5). Blood samples for haematocrits were taken at regular intervals (see results) after transfer, until blood p.c.v. returned to original values. In order to keep the total blood sample volume from each fish as small as possible, osmolarity was only measured at the beginning of the experiments, after 3 days, and at the end. The second group of 5 animals contained 2 which had been unfed for over 14 days and showed hypo-osmotic serum. All

5 were exposed to some of the diluted sea water which contained sufficient phenol red to colour it deep pink. After 24 h in dyed medium, gut fluid was sampled for traces of phenol red, as described previously.

In a third group of 5 fish, 3 normally-fed animals and 2 unfed ones were transferred to a hyperosmotic medium (Table 6). Animals were treated exactly as the above-mentioned group, with the phenol red being added to the hyperosmotic medium.

BRANCHIAL ION EXCHANGE

Five fish were used for the determination of branchial chloride exchange by means of radio-isotope. Each fish was anaesthetised in sea water containing 0.03% MS-222 and was then injected intravenously with about 3 μCi of Chloride-36 in isotonic saline. The fish was then replaced in the sea water containing MS-222 for a further 15 minutes, to ensure complete anaesthesia and cessation of any superficial bleeding from the injection. It was then placed upside down on the sloping Plexiglass table of the experimental apparatus (Fig. 9) and 1 litre of fresh sea water, containing just enough MS-222 to maintain light anaesthesia, was recycled over the gills. After being sprayed into the gill arches, from lateral jets inside the buccal cavity, the sea water ran out through the gill slits, thus irrigating the gills, and was collected in a reservoir below, from where it was recycled and oxygenated. The posterior part of the fish was bathed continuously in a small volume of sea water separated from the recycling anterior compartment. After 6 to 10 h, samples of the recycled sea water were taken and the fish returned to its

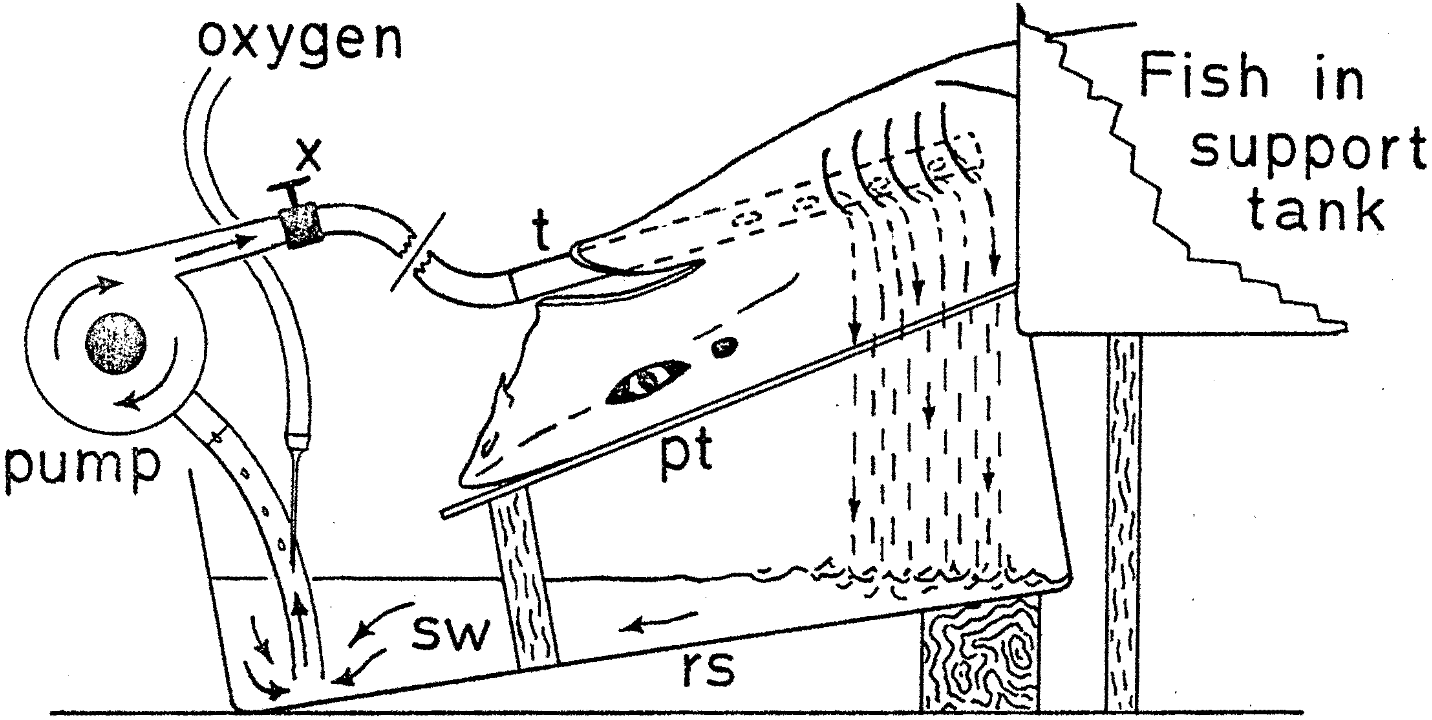

FIGURE 9. Apparatus used for determination of branchial ion exchange. Sea water (sw) is pumped up from the reservoir (rs), flow rate being controlled by tap X, and into a tube (t) with lateral jets, inserted into the buccal cavity of the fish. The animal's head is inclined on a Plexiglass table (pt), so that water flows out of the gill slits and back to the reservoir, for recycling. Equipment shown in Plates 5 and 6.

PLATE 5. Apparatus used for determination of branchial ion exchange showing Plexiglass table (centre) above glass reservoir. Oxygen line (background) can be seen, ending in hypodermic needle entering sea water uptake pipe above plastic 'window'.

PLATE 6. Apparatus used for determination of branchial ion exchange showing fish in position with drugged sea water being pumped over the gills. The posterior half of the fish is supported and bathed in the cut-away support tank (background). Oxygen needle can be seen in the left foreground.

laboratory tank. The average activity of 6 samples of original (non radioactive) sea water was compared with that of 6 samples of the final recycled medium, in each experiment. Results were analysed statistically.

Sodium loss at the gills was determined by a similar method, for 3 fish injected with Sodium-22 radio-isotope.

The above technique was only carried out with fish in normal sea water (467 mM Na/l, 550 mM Cl/l) and was used merely to determine whether or not ions were being lost at the gills. In order to measure the rate of ion loss (i.e. efflux rate), it is appreciated that the technique requires further modification.

SURGICAL METHODS

Five fish (plus 1 control animal) were used to investigate the effects of inactivation of the urinary and rectal gland systems upon blood sodium and chloride levels. The experiment was carried out on animals in approximately normal salinity sea water.

Each fish was anaesthetised in sea water containing 0.03% MS-222 for 15 minutes and was then transferred to an inclined Plexiglass operating table and was arranged ventral surface uppermost and the head lowest. Sea water, cooled by ice packs and containing sufficient MS-222 to maintain anaesthesia, was then recycled over the gills, from a reservoir below.

Under anaesthesia, the urinary opening was closed by a "purse string" suture around the neck of the urinary papilla and the arrangement was checked, by drying the papilla and squeezing the urogenital tract gently (to stimulate urination). The ventral surface of the abdomen was then opened by a small mid-line incision, just anterior to the pelvic fins. The duct joining the rectal gland to the gut was then tied off securely with 2 silk sutures, as near to the gland as possible. The peritoneum and body wall were then sutured, after the addition of a small amount of antiseptic cream to the wound. The suture was made waterproof by the addition of water-repellant cream to the outside.

Capillary bleeding during surgery was eliminated by immediate cauterization and infection risk was kept minimal by normal surgical aseptic techniques. In all cases, infection of the wound was not apparent for more than 14 days after surgery.

After operation, fish were allowed to recover in experimental tanks heavily shaded to reduce activity of the fish. Blood samples were taken from the fish at intervals (see results) over a period of 2 weeks.

HISTOCHEMICAL EXAMINATION OF GILLS

Histochemical examination of gill tissue was carried out by light microscopy, after silver staining, by Copeland's (1948) modification of the Leschke method. This test for chloride has been previously used at the light microscope level by Copeland and Datta Munshi (1964) and more recently by Fearnhead and Fabian (1971), at the electron microscope level.

The test was carried out on 3 fish from high-salinity sea water (740 mM Cl/l), 3 fish from normal sea water (550 mM Cl/l) and 3 fish from diluted medium (450 mM Cl/l) - all of which had been normally fed. In addition, 2 animals with urinary and rectal gland systems sutured, were examined after the experimental period of 2 weeks. Two animals with hypo-osmotic serum, which showed traces of phenol red in the gut, were also sacrificed for the chloride test.

Animals were removed from sea water, stunned with a sharp blow to the head and quickly decapitated. Portions of each gill arch were then excised and immediately transferred to isotonic saline at 5°C. Under saline, filaments were cut free and after briefly washing under distilled water (to remove surface chloride), were subjected to silver staining. This was originally carried out after Copeland (1948), with gold toning, to increase contrast, but later modified to the following procedure.

Silver staining :

1.	Fixation	18 h in a solution made up of 1 gm silver nitrate, 2 ml of concentrated nitric acid and 98 ml distilled water. (Half of the tissue in the first experiment was used as control, and subjected to a similar solution but without the silver nitrate.)
2.	Washing	2 x 15 minutes in distilled water.
3.	Developing	20 minutes in Kodak Microdol-X developer, diluted with 3 parts distilled water.
4.	Washing	2 x 5 minutes in distilled water.
5.	Fixation	20 minutes in M&B Amfix, diluted with 3 parts distilled water.
6.	Washing	6 x 30 minutes in distilled water.

With the exception of the final washing (stage 6), silver staining was carried out in total darkness, with only indirect illumination during transfer of tissue from one solution to the next. After staining, tissue was subjected to gold toning, carried out in subdued light, as follows.

Gold toning

1.	Toning	10 minutes in a solution of 1% chloroauric acid, containing 3 drops of glacial acetic acid per 100 ml.
2.	Washing	2 x 1 minute in distilled water.
3.	Developing	5 minutes in 2.5% oxalic acid.
4.	Washing	2 x 1 minute in distilled water.
5.	Fixing	10 minutes in M&B Amfix, diluted with 3 parts distilled water.
6.	Washing	2 x 30 minutes in distilled water.

Tissue was then dehydrated through a series of increasingly concentrated alcohols, in the normal manner, and was cleared in xylene. Tissue was then mounted in paraffin wax (m.p. 52°C) for section cutting. Longitudinal and transverse sections of filaments and their bases were cut, with a thickness range of 5 to 10μ, with a Leitz microtome. Sections were then de-waxed, rehydrated and stained for 1 h in 3% potassium dichromate solution. Sections were then removed and, after rinsing briefly under distilled water, were stained for 1 minute in a solution of alum-haematoxylin and eosin in 20% alcohol. Counter-staining was carried out for 3 minutes in a solution of light green in 10% alcohol. Sections were then dehydrated, cleared and mounted in Depex, in the normal manner. Sections were examined by light microscope and photographed in Ilford Pan F film, using a Zeiss 1540 Photomicroscope 11. The film was developed with a Beutler developer (K.Oates - personal communication) to increase tonal range.

FLUID MEASUREMENTS

All blood samples for serum analysis were allowed to clot overnight, in glass centrifuge tubes, at $5^{o}C$. After serum separation by centrifuging, osmolarity was read with a Knauer platinum-thermistor osmometer (accuracy ± 5 mOsm/l), chloride after the method of Schales and Schales (1941) (accuracy ± 5 mM/l) and sodium by flame emission spectroscopy, using a Unicam SP 1900 Atomic Absorption Spectrophotometer (accuracy ± 5 mM/l).

Chloride-36 activity in sea water was assayed after dissolving 0.5 ml sea water in 5 ml of Beckman TLA "Fluoralloy"/Triton X-100 scintillation mixture, for each sample. Samples were counted on a Packard Tricarb Model 3380 Liquid Scintillation Spectrometer, using an Automatic External Standardization system for quench corrections. Sodium-22 activity was measured directly with 5 ml sea water samples, using a Packard Model 578 Gamma Scintillation Spectrometer.

RESULTS

HAEMATOCRITS

P.c.v. results are presented in Tables 4 to 6. Table 4 shows the effect of medium dilution upon 6 normally-fed fish. Tables 5 and 6 each show the effect of change in external medium upon 3 normally-fed and 2 underfed fish with hypo-osmotic serum.

Average p.c.v. drop, based on the total of 9 normal fish (Tables 4 and 5), exposed to medium dilution, was 19.9 ± 4.9%, whereas average p.c.v. rise, for 3 normal animals exposed to medium concentration was only 13.6 ± 2.4%. Since only 2 hypo-osmotic animals were examined in each transfer (Tables 5 and 6), the % p.c.v. changes were not averaged.

DRINKING OF THE MEDIUM

Phenol red (visibly concentrated) was found in stomach fluid of all experimental animals. Four of them showed slight traces of the dye in intestinal fluid. No intestinal fluid was obtained from the other 2 experimental animals. The 3 control fish showed no traces of dye in either stomach or intestinal fluid.

Table 7 presents serum osmolarities of the 9 animals, at the time of experimentation.

Phenol red also found to be concentrated in the stomach fluid of the 4 hypo-osmotic dogfish exhibiting large p.c.v. changes (Tables 5 and 6). No intestinal fluid was obtained from these fish.

Table 4. Six normally-fed fish exposed to dilution of external medium.

		p.c.v.'s (% total blood volume)					
	Fish	1	2	3	4	5	6
Medium	1000 mOsm/l	22.5	24.0	11.0	19.0	22.0	10.5
New medium.	880 mOsm/l						
	1 day	16.0	20.0	9.0	16.5	19.0	9.5
	2 days	16.5	20.5	9.0	16.0	17.0	9.0
	3 days	18.0	21.0	9.5	17.5	17.5	9.5
	6 days	22.0	23.5	11.0	18.5	21.5	10.0
	9 days	22.0	23.5	11.0	19.0	22.0	10.5
Max. % p.c.v. change for medium dilution of 120 mOsm/l		-28.8%	-20.0%	-18.0%	-14.7%	-13.6%	-14.3%
Serum osmolarities (mOsm/l)							
Before transfer		1025	1020	1030	1030	1025	1030
	3 days	895	900	905	910	900	905
	9 days	890	900	905	900	900	900

Table 5. Three normally-fed fish and 2 hypo-osmotic ones (Nos. 4&5) exposed to dilution of the external medium.

	p.c.v.'s (% total blood volume)				
Fish	1	2	3	4	5
Medium 1000 mOsm/1	23.5	23.0	12.0	22.0	27.0
New medium 900 mOsm/1					
2 days	18.0	18.0	9.0	13.0	15.0
4 days	21.0	18.0	9.0	14.0	19.0
6 days	23.0	20.0	10.0	16.0	22.0
9 days	24.0	23.0	12.0	20.0	24.0
11 days	24.0	23.0	12.0	21.0	26.0
Max. % p.c.v. change for medium dilution of 100 mOsm/1	-23.4%	-21.7%	-25.0%	-40.9%	-44.5%
Serum osmolarities (mOsm/1)					
Before transfer	1030	1025	1025	980	970
3 days	935	935	920	890	885
9 days	925	920	920	880	875

Table 6. Three normally-fed fish and 2 hypo-osmotic ones (Nos. 4&5) exposed to concentration of the external medium.

		p.c.v.'s (% total blood volume)				
	Fish	1	2	3	4	5
Medium	900 mOsm/l	21.0	10.5	19.0	12.0	13.0
New medium	1010 mOsm/l					
	1 day	22.0	10.5	20.0	14.0	17.0
	3 days	24.0	12.5	21.0	14.0	17.0
	6 days	22.0	12.0	20.0	16.0	18.0
	9 days	21.0	11.0	19.0	14.0	17.0
	11 days	21.0	11.0	19.0	13.0	14.0
Max. % p.c.v. change for medium concentration of 110 mOsm/l		+14.2%	+16.3%	+10.5%	+33.3%	+38.0%
Serum osmolarities						
	Before transfer	915	920	910	890	890
	3 days	1010	1020	1010	980	990
	9 days	1025	1030	1030	980	980

Table 7. Serum osmolarity of 6 hypo-osmotic animals and 3 hyperosmotic control fish, at the time of treatment with phenol red.

Fish	Control 1	C2	C3	1	2	3	4	5	6	Sea water
serum (mOsm/1)	965	970	970	920	910	930	930	920	920	950

Table 8. Serum osmolarity (Om = mOsm/l) and sodium and chloride concentrations (mM/l) for 5 fish with inoperative urinary and rectal gland systems and 1 control animal. *indicates point at which urinary sutures were released from 3 animals resulting in returned urine flow.

	Fish 1			Fish 2			Fish 3			Fish 4			Fish 5			Fish 6 (Control)		
Days	Cl	Na	Om	Cl	Na	Om	Cl	Na	Om	Cl	Na	Om	Cl	Na	Om	Cl	Na	Om
	280	270	1020	275	271	1010	248	250	970	258	239	960	256	247	970	248	235	970
1																		
2							280	291	970	264	261	960	260	260	970			
3																		
4	310	298	1020				288	295	980	272	-	970	288	265	975			
5																		
6				325	320	1015				304	300	980	300	-	980			
7	330	329	1020				310	305	1010							245	237	970
8										*			300	285	980			
9				344	326	1015	*			280	260	970	*					
10							305	296	1000									
11	335	330	1025	345	325	1015				270	256	960	270	260	-			
12							300	-	1000									
13																		
14				348	327	1015	300	261	980	240	250	955	260	252	970	248	236	970
15																		

Sea water	Cl	Na	Om
	565	462	1000

Sea water	Cl	Na	Om
	525	445	950

BRANCHIAL ION EXCHANGE

The 5 experimental fish injected with Chloride-36 showed differences between activities of final sea water samples and initial sea water samples which were statistically significant ($p<0.001$) in every case.

The same was true for the 3 fish injected with Sodium-22.

SURGICAL METHODS

Serum osmolarity, and sodium and chloride concentrations of 5 fish (plus control), with inoperative urinary papillae and rectal glands are recorded over a 14-day period, in Table 8.

Since several blood samples were taken from each fish, it was necessary to keep sample volumes down to 0.7 ml each time, with the possibility of only one measurement of osmolarity, sodium and chloride concentration per sample. However, previous experiments have shown that experimental error is sufficiently small for one reading to be representative of the true value, to within the stated accuracy.

HISTOCHEMICAL EXAMINATION OF GILLS

The nature of the chloride test is such that all chloride ions in the tissue react with silver nitrate to form silver chloride deposits, which are then developed photographically to silver. These brownish deposits are then darkened further by gold toning, so that finally all concentrations of chloride in the tissue appear as black granular deposits.

In normal sea water (550 mMCl/l) and higher salinities, gill tissue showed abundant silver deposits distributed both at the bases of secondary lamellae and throughout epithelial cells on the lamellae. The low-power appearance of the gills is shown in Plate 7. A similar appearance was shown by gill tissue from 2 hypo-osmotic fish, which were found to be drinking the medium, and from 2 animals with sutured urinary and rectal gland systems.

Transverse sections of primary lamellae showed definite intracellular silver deposits in cells surrounding the afferent and efferent blood vessels - in the positions where "chloride cells" were reported by Copeland (1948), as shown in Plate 8.

The total absence of silver deposits in gill tissue from fish in diluted medium (450 mMCl/l) is illustrated in Plate 9.

Plates 10 and 11 show the intracellular silver deposits under higher magnification, as they appear at the bases of the secondary lamellae, in the positions where "chloride cells" have been reported by Datta Munshi (1964).

By the nature of the chloride test, these silver deposits can be considered representative of original intracellular chloride concentrations or deposits and estimation of the size of these chloride deposits suggests a 5 to 15 µ diameter.

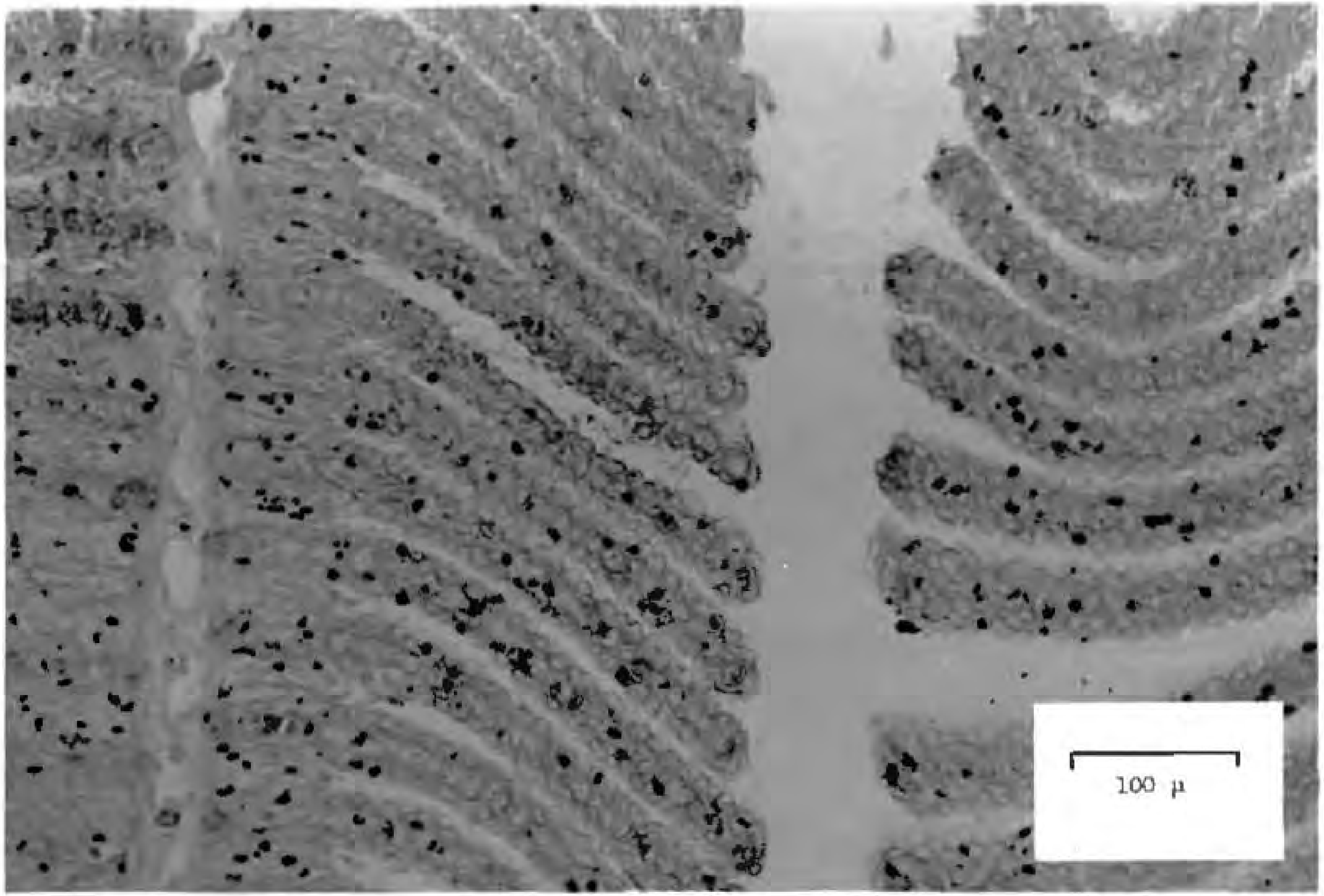

PLATE 7. Low-power appearance of gill tissue after silver staining, showing deposits at the bases of secondary lamellae and in epithelial cells throughout the lamellae.

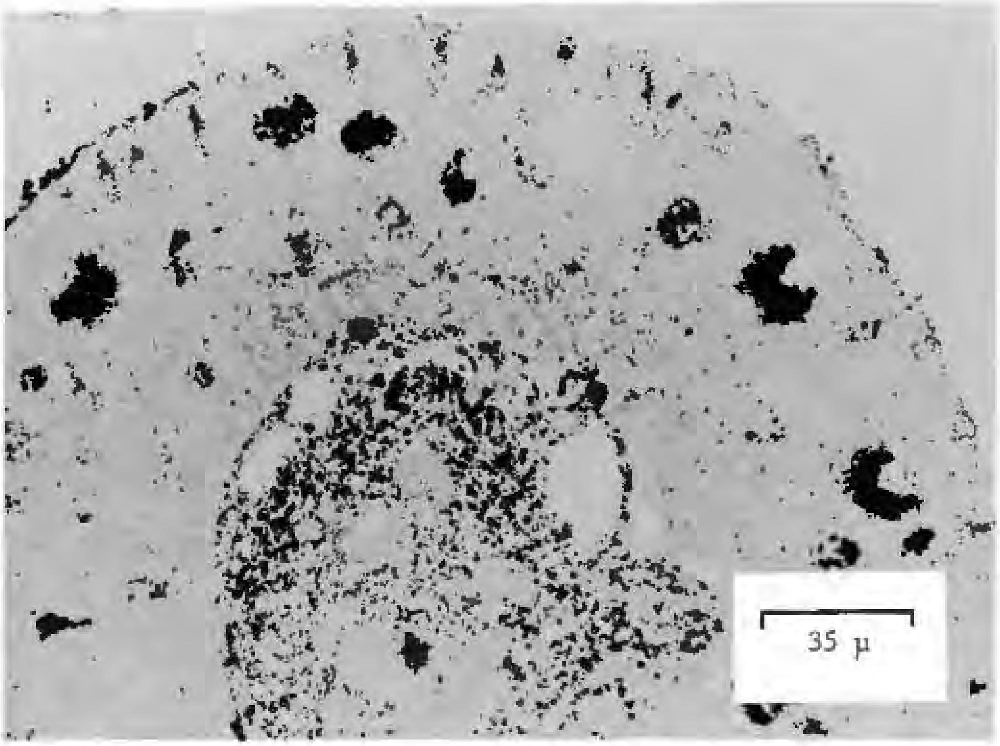

PLATE 8.

PLATE 9.

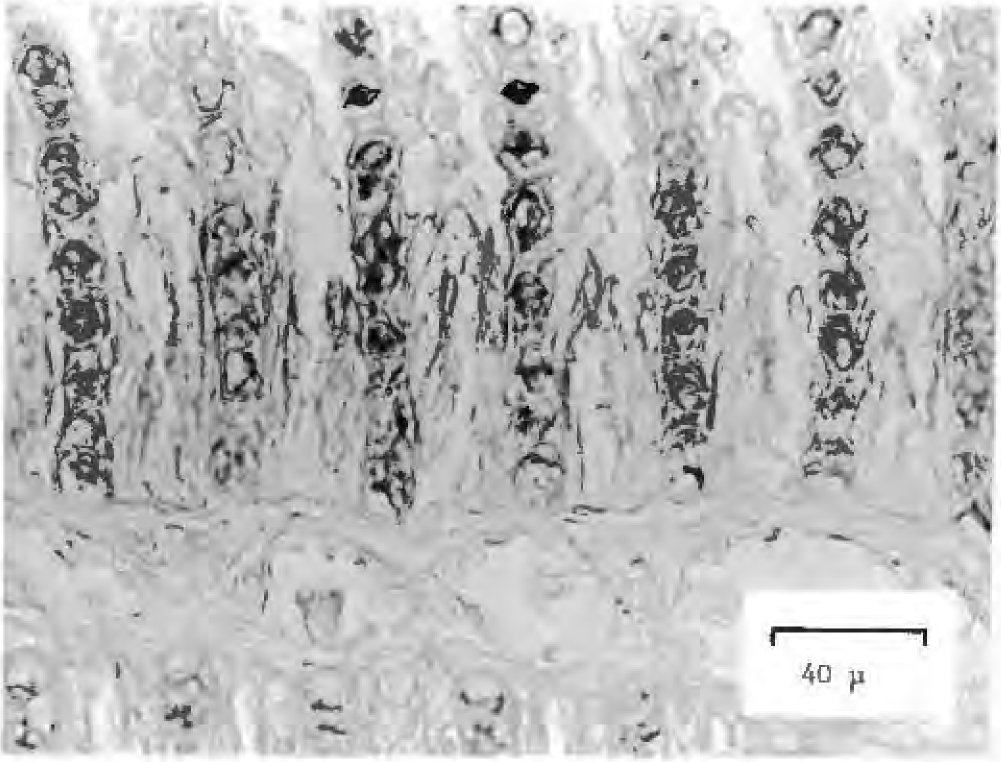

PLATE 8. Transverse section through a primary gill lamella, (after silver staining), showing intracellular silver deposits (black patches) in cells surrounding the afferent and efferent blood vessels.

PLATE 9. Secondary gill lamellae from fish in dilute medium, showing absence of intracellular silver deposits after silver staining.

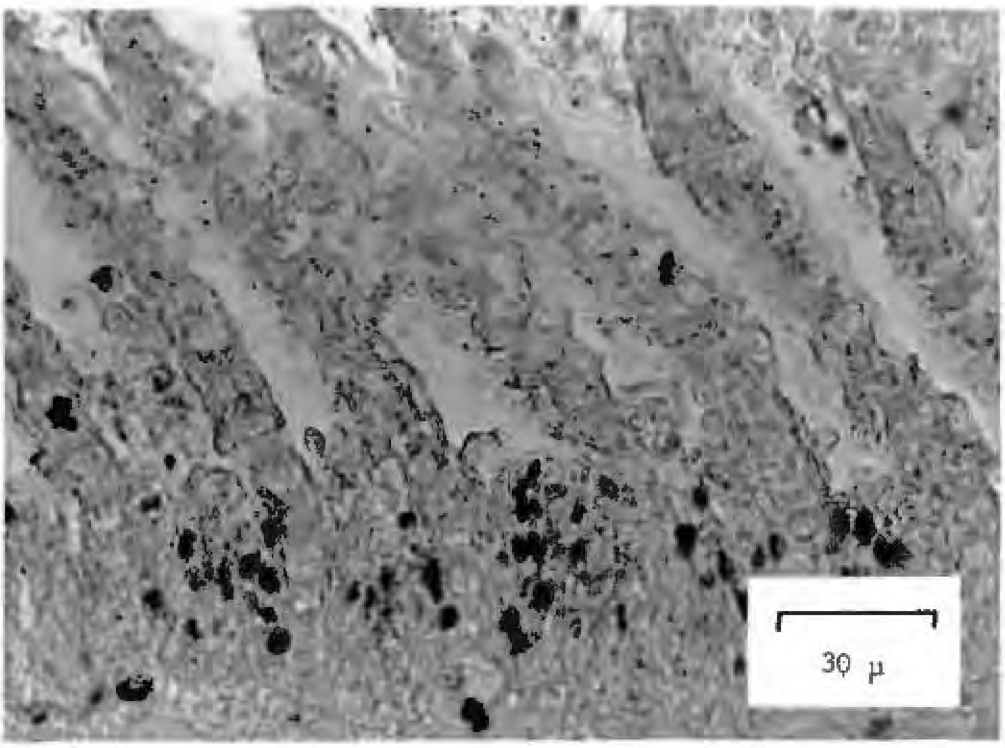

PLATE 10.

PLATE 11.

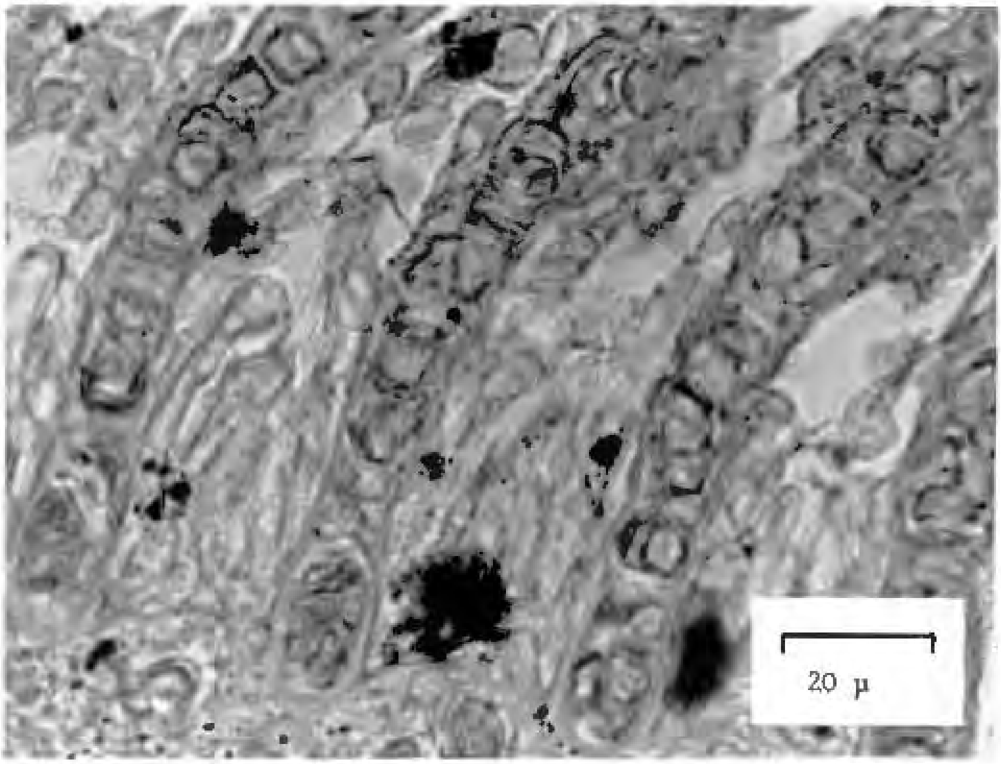

PLATE 10. Abundant silver deposits between the bases of secondary gill lamellae, (after silver staining), suggesting concentration of chloride ions in positions where "chloride cells" have been reported by Datta Munshi (1964).

PLATE 11. Interlamellar silver deposits under higher magnification.

DISCUSSION

Haematocrit results for *Poroderma africanum* indicate both a high sensitivity to external osmolarity and permeability to water - a factor which has been previously demonstrated (Haywood, 1974a). After 3 days in new medium, the osmolarity of the serum rapidly approached that of the new medium. These results suggest rapid drop in serum osmolarity immediately following dilution of the external medium. If such a drop were to "overshoot" the dilution of the medium, it could account for the quick stabilization of blood values, since water gain would be stopped and equilibrium re-attained.

Changes in p.c.v. and sensitivity to variations of the external medium vary with different elasmobranch species. Goldstein and Forster (1971) showed a 20% drop in blood haematocrits for the little skate *Raja erinacea*, when transferred over 4 days from full to approximately half-strength sea water. Similarly, de Vlaming and Sage (1973) have shown that haematocrits decreased for stingrays (*Dasyatis sabina*) when transferred to diluted sea water, but returned to normal within 6 days, in the new medium. Both the above species showed gain in weight upon transfer to diluted medium, indicating a net water influx. The return of p.c.v.'s to original values, within 6 or 7 days, indicates that body water content was regulated at a constant level. Goldstein *et al*. (1968) showed, however, that lemon sharks (*Negaprion brevirostris*), on transfer over a period of 1 week, from full to approximately half-strength sea water, showed no decrease in p.c.v. values and they concluded that sharks in a diluted medium maintained water balance.

The use of phenol red dye demonstrated that normally-fed dogfish do not drink the medium. Water influx, resulting in p.c.v. drop, therefore seems most likely to occur at the gills. Fish which were found to be drinking the medium, showed more pronounced changes in p.c.v., upon alteration of the external medium. Furthermore, p.c.v.'s of the hypo-osmotic animals did not approach original values again until about 11 to 13 days after transfer to the new medium - suggesting that the augmented water flux between fish and sea water, due to drinking of the medium, had a pronounced effect on the water balance of the species.

Motais _et al_. (1969) have shown that in eels, most of the diffusional water flow occurs at the gills and Evans (1969) showed that euryhaline teleosts appear to have greater permeability to water when in dilute medium, than when in sea water. This change in permeability is due in part to the lower concentration of calcium in fresh water, compared with sea water, and also to the higher concentration of the hormone prolactin in freshwater-adapted fish (Potts and Fleming, 1970). Previous results (Haywood, 1974a) have shown that water permeability, as measured by tritiated-water turnover, appears to be maximal in normal sea water, declining somewhat with either dilution or concentration of the medium. Temperature effects upon water permeability have been previously investigated and Evans (1969) gave a Q_{10} for teleostean water flux as 1.9. Motais and Isaia (1972) have shown a temperature dependance of diffusional permeability to water in freshwater-adapted and seawater-adapted eels. In the former group, they found that the osmotic permeability was considerably higher than the diffusional permeability, indicating the presence of water-filled channels in the branchial epithelium. The reverse situation occurred for seawater-adapted eels, suggesting that either the osmotic pressure difference between blood and external medium did not represent the true osmotic gradient across the tissue, or that absorption of water, linked with a movement of solutes, was occurring at a specialized region of the gill.

Payan and Maetz (1971) have shown very high water permeabilities at the gills, in the three elasmobranchs, Scyliorhinus canicula, Raja montagu and Torpedo marmorata. "Diffusional permeability at the gills was measured with the help of tritiated water, by evaluating the rate of water exchange between internal and external media, in the above three cases. The rate was found to be very high, amounting to 157, 167 and 97%/h respectively." Their results were obtained at 16°C - slightly higher than present experimental temperature. It was shown by these two workers, that the gill was the major site of water exchange, and that the cardiac output, measured by applying Fick's principle in branchial tritiated water clearance, was not a limiting factor, for the determination of diffusional water flow at the gills.

Whilst no assessment of branchial urea exchange was made in this investigation, it has been previously considered that the elasmobranch gill is fairly impermeable to urea and hence little branchial urea exchange might be expected. However, recent investigations have tended to argue against this. Boylan (1967) found that branchial urea excretion in Squalus acanthias was about 25 µM/100g/h at 16°C. Measurements of branchial urea exchange in the euryhaline skates, Raja erinacea and R. radiata (Payan et al., 1973) showed an output of between 19.8 and 57.0 µM/100g/h, depending on external salinity. They found that output increased at higher salinity, due to the higher serum urea level, rather than change in actual branchial permeability. Goldstein and Forster (1971) found a branchial urea excretion of around 23 µM/100g/h for skates of the same species in 100% sea water, but the difference between their results and those of Payan _et al_. may have been due to the fact that the latter workers fed their animals during the experiments, whereas Goldstein and Forster did not. Serum urea levels might well have been different in the two sets of fish, due to this, as occurs in the dogfish, _P. africanum_ (Haywood, 1973). The results of Payan _et al_. (1973) have shown that branchial urea output is far in excess of renal output and indeed must contribute greatly to the overall urea loss.

Present results with Chloride-36 and Sodium-22 indicate that both ions are lost at the gills, against a concentration gradient. Whilst this in itself is not evidence of active transport at the gills and it is appreciated that a certain degree of passive branchial efflux may be accounted for by Fick's principle, such findings are nevertheless consistent with a suggestion of branchial transport of ions, as a possible regulatory mechanism in _Poroderma africanum_. Maetz and Lahlou (1966) showed that for the dogfishes _Scyliorhinus canicula_ and _S. stellare_ both urinary and rectal gland salt outputs were minimal by comparison with branchial output. Horrowicz and Burger (1968), measuring sodium fluxes across the head region of the spurdog, _Squalus acanthias_, concluded that ion movement through the skin itself only accounted for 2 to 3% of the total sodium influx. They concluded that sodium influx through the gills was about 10 times that through the skin, although in this case, the ion movement was along the concentration gradient. Above findings, together with those of Burger and Tosteson (1966), suggest that the head region, and in particular the gills, of S. acanthias may be a source of ion influx - probably by passive diffusion along the ionic gradient. Present results suggest that such passive influx of ions at the gills is only a part of a more complex ionic turnover at this site, where ionic efflux at least balances and probably exceeds the diffusional influx. Similar conclusions may be drawn from the findings of Payan and Maetz (1970), where branchial salt regulation in the genus Scyliorhinus was found to account for two-thirds of the total sodium efflux. By comparison, rectal gland secretions were minimal, providing only one-tenth of the total sodium efflux. More recently, Payan and Maetz (1973) have measured branchial sodium transport mechanisms in Scyliorhinus canicula, and have suggested the presence of a Na^+/NH_4^+ and Na^+ H^+ exchange mechanism, which bears strong similarities to mechanisms previously shown to act in teleost gills (Maetz and Garcia Romeu, 1964; Maetz, 1971, 1972; de Renzis and Maetz, 1973; Whitelaw, 1973). Sodium exchange at marine teleost gills has been shown to be temperature dependant (Maetz and Evans, 1972), as would be expected for any active transport mechanism and it therefore seems probable that the models proposed by these workers, for ion transport across teleost gills, may have their counterpart in the gills of elasmobranchs. Payan and Maetz (1973) felt that it was unwarranted to definitely attach an active transport mechanism to their findings of sodium transfer, without information on the electrical potential across the gill epithelium, as has been reported in eels (Maetz and Campanini, 1966), but they still felt that, by ion transport, the gills played a major role in the osmo-regulation of the species.

In keeping with the findings of Maetz and Lahlou (1966), present results suggest that the rectal gland plays a minor role in salt regulation in P. africanum. Surgical closure of both the rectal-gland duct and urinary papilla caused initial rise in serum sodium and chloride levels, but in all cases the rise stopped after about 1 week, suggesting compensatory regulation by some other organ. Release of the urinary constriction, after about 8 days, in 3 animals, resulted in a fall in blood sodium and chloride levels, until they approached initial values. Such animals, with non-functioning rectal glands, were kept successfully in the laboratory, without appreciable rise in serum sodium and chloride levels, for over 14 days. These results are interpreted as indicating a greater regulatory mechanism by the kidneys and gills than by the rectal gland. Subsequent unpublished results further support this idea, suggesting that salt regulation by the rectal gland is minimal in P. africanum.

Histochemical examination of gill tissue from 2 animals treated as above showed abundant chloride concentrations between the bases of secondary lamellae and also in many of the epithelial cells around the margins of the secondary lamellae (Plates 7, 10 and 11). Such findings argue for chloride transport by the gill epithelium and, in the light of the previous results, suggest that when urinary and rectal gland salt regulation is inoperative, increased salt regulation at the gills may be sufficient to maintain blood salt levels, over the time period examined. Present results have also shown a salinity effect upon the abundance of branchial chloride concentrations in normal fish. Animals in dilute salt sea water (450 mMCl/l) showed no branchial chloride concentrations (Plate 9) whereas fish from normal sea water (550 mMCl/l) and hyper-saline medium (740 mMCl/l) showed abundant branchial chloride concentrations (Plates 7, 8, 10 and 11). Such results suggest an increased chloride transport by the gills in higher salinities - an observation which has been made on teleost gills by Liu (1942), who found that the development of "chloride cells" was favoured by increasing external salinity. Such was also shown in the eel Anquilla rostrata by Getman (1950).

The "chloride-secreting cells" found by Keys and Willmer (1932) in the gill tissue of the common eel, were considered to lie mainly in the bases between the secondary lamellae, although "isolated cells of the same character have been observed occasionally on the outer regions of the leaflets". They discerned the cells as being quite separate from mucous cells, being located in such positions as to be in close proximity to both blood and external medium. In the gills of a 250 g eel, they estimated the presence of between 3 and 6 million such cells, but interestingly they found these cells to be generally less abundant in fresh-water animals than in the marine forms. In Scyliorhihus canicula, the only elasmobranch they examined, they found no "chloride cells", although mucous cells were abundant around and between the secondary leaflets.

Krogh (1937) felt that these "chloride cells" were able to reverse their action, for fish in fresh water, where they could actively take up chloride ions from the external medium. Since then, many reports of this ability by the gills of fresh-water fish (Datta Munshi, 1964; Kerstetter and Kirschner, 1972; Maetz, 1972; de Renzis and Maetz, 1973; Whitelaw, 1973) have suggested an active transport mechanism, acting inwards for fish in fresh water. Copeland's work (1948) on the euryhaline teleost Fundulus heteroclitus, supported the Keys-Willmer theory and Copeland and Pettengill (1948) together demonstrated that enzyme activity could be localized within these cells. Such has also been shown in "chloride cells" of euryhaline teleosts (Kamiya, 1972), thus providing further evidence of an active transport mechanism for chloride transfer.

Not all workers were satisfied with the Keys-Willmer theory of definite "chloride cells" however, and Bevelander (1936, 1946), after a comparitive study of many fish, suggested that the "chloride-secreting cell" was in fact a mucous cell. Later, Vickers (1958) suggested that these cells represented metaphasic forms of mucous cells. The various ideas on these cells, by several workers, are discussed by Parry et al. (1959) but the general trend seems to be that cells with a definite "chloride-secreting capacity" do exist in many fish gills, whether they are specially developed cells or modified mucous cells of the epithelium.

Whilst there is much evidence concerning chloride cells in teleosts, they had not been found in elasmobranchs until 1961, when Doyle and Gorecki demonstrated their presence, by electron-microscopic preparations of the gills of 6 genera of fish, including a marine elasmobranch (Urobatis), a euryhaline species (Fundulus) and a primarily fresh-water form (Notropis). They reported that "in some forms, this cell is in contact with the environment, and in others, it is covered by other epithelial cells". They were however, doubtful as to its supposed function as the principal site of chloride transfer. Later work on the S-cells of the guppy, Lebistes reticulatus (Strauss, 1963) also suggested that these "so-called chloride cells" seemed unlikely to be concerned only with osmoregulation. Fearnhead and Fabian (1971), with another electron-microscopic gill examination, this time of the euryhaline teleost *Monodactylus argenteus*, observed definite chloride cells and supported the Keys-Willmer theory regarding its ion-secretory role.

Payan and Maetz (1973) have reported the presence of "chloride cells" in the gills of the genus *Scyliorhinus* and have further supported an active-transport theory by their measurements of branchial sodium transport in this species. Present results also suggest that such cells, or at least modified cells with this capacity, are present in *Poroderma* gill tissue. However, in view of the controversy which still seems to exist, as to whether the ions are put out by definite and specialized "chloride cells" or by modified epithelial mucous cells, and since histochemical examination of *Poroderma* gill tissue was only carried out at the light microscope level, it is felt that the term "chloride cell" should be avoided, with specific reference to *P. africanum*. Since intracellular chloride concentrations were found in cells lying between the secondary leaflets and around their margins, where these cells were equally in close contact with the blood and external medium, and in view of the increased number of such cells, at higher salinities, and under conditions when greater salt regulation would be expected by the gills, it seems most probable that such cells are concerned with ion transport, and their presence is considered to constitute a definite regulatory capacity to the gills, although the cells may not be specialized "chloride cells".

Hypo-osmotic specimens of *P. africanum*, which were shown to be drinking the medium, also exhibited branchial chloride concentrations suggesting that an unknown fraction of the extra ingested salt is probably excreted by the gills. Indeed, under such conditions, urine flow would be reduced, with resultant drop in renal salt excretion so that the gills might have to handle some of the load normally put out by the kidneys. No results are at present available to indicate how the rectal gland is affected under such conditions.

In conclusion therefore, it seems that similar branchial salt balance mechanism exist in both marine teleosts and the marine elasmobranchs so far examined. From an evolutionary point of view, it now seems possible that living elasmobranchs have retained branchial mechanisms inherited from their fresh-water ancestors - mechanisms which later became well modified in the teleosts, but which nevertheless are still functional in the elasmobranchs.

CHAPTER 7

UNUSUAL EFFECTS OF SALINITY CHANGE UPON SERUM SODIUM LEVELS.*

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* Manuscript submitted to 'Journal of Experimental Marine Biology and Ecology' July 1974.

ABSTRACT

The effects of salinity change upon serum sodium were investigated, under laboratory conditions, in the striped dogfish *Poroderma africanum* (Gmelin). Animals were found to tolerate an external sodium concentration range of 280 - 665 mM/1. At the extremes of this range the blood sodium levels were approximately 190 and 365 mM/1 respectively - with a blood value of 238 mM/1 in normal sea water. No effect of feeding frequency upon blood sodium was observed, and both sexes produced similar results. Comparison of present findings with previous blood chloride results, obtained under similar conditions (Haywood, 1973), showed that serum sodium was lower than serum chloride in all mixohaline media up to normal sea water concentration (approx. 1030 mOsm/1, 550 mM Cl/1 467 mM Na/1), but above about 107% sea water, serum sodium rose sharply, exceeding serum chloride. Blood potassium levels were also measured in fish exposed to 3 different salinities. Animals were also exposed to non-mixohaline media, produced artificially in the laboratory. Results from such fish suggest that blood sodium and chloride are regulated independantly of each other, but proportionally to the concentration of the particular ion in the external medium. Results are compared with previous relevant findings of other workers.

INTRODUCTION

As in marine teleosts, the blood of marine elasmobranchs is generally found to be about half the tonicity of sea water, so that sodium chloride concentration is roughly in the region of 250 - 280 mM/1, depending on the species. The main difference between elasmobranch and teleost blood is the high urea concentration present, a common factor to most elasmobranch body fluids and tissues, and one which is due largely to renal reabsorption and retention of urea (Kempton, 1953; Boylan, 1967, 1972). The osmotic strength of the urea makes up the osmotic deficit which would otherwise exist between internal and external media. Consequently, whilst the blood of marine elasmobranchs shows only about half the salt concentration of sea water, its osmotic strength is usually equal to, or a little greater than that of the sea water. Hence it has been considered (Smith, 1931) that marine elasmobranchs do not drink the medium, as do their teleost counterparts. Such is normally the case, for most elasmobranchs, although it has previously been shown (Haywood, 1974b) that underfed individuals of Poroderma africanum, exhibiting hypo-osmotic blood, drink the medium in appreciable quantities to balance osmotic efflux.

The effects upon blood salt levels, produced by varying the external salinity, have been investigated for a few species of marine elasmobranchs. Evidence that salinity change was producing either a concentrating or diluting effect upon elasmobranch blood was demonstrated by early workers (Scott, 1913; Chaisson, 1930; Margaria, 1931) who showed that, when an elasmobranch is placed into diluted sea water the overall osmolarity of its blood falls and, conversely, rises when the fish is exposed to concentration of the external salinity. It was deduced that this change in overall osmolarity was produced by a change in the concentration of the various ionic and osmotic components. Later, Price & Creaser (1967) showed that exposure of the skate Raja eglanteria to low salinity produced a depression of the serum chloride level, although not by a great amount. Burger (1962, 1965) has stated that blood chloride level in the spiny dogfish Squalus acanthias is resistant to dilution of the medium, but then, rather surprisingly, indicated that dilution of the external medium could cause a drop from about 250 mM/1 to 220 mM/1 in blood chloride concentration. More recently, Goldstein and Forster (1971) have shown that variation of the external salinity produces effects upon the little skate Raja erinacea similar to those shown by Price and Creaser. Similar findings were also reported for the stingray Dasyatis sabina (de Vlaming & Sage, 1973) and for the lemon shark Negaprion brevirostris (Goldstein et al., 1968). An overall salinity change, of from approximately 18.0‰ up to 47.5‰ has previously been shown to produce a change in serum chloride, from about 200 - 315 mM/1, in the striped dogfish Poroderma africanum (Haywood, 1973) - but no previous salinity-change effects on serum sodium have been studied.

The only previous measurements of serum sodium, for Poroderma africanum, were in normal sea water. At this salinity, serum sodium was found to average 238 mM/1 (Haywood, 1974a), whereas serum chloride was 261 mM/1 (Haywood, 1973). In many marine elasmobranchs the blood sodium level is higher than that of chloride and therefore these findings seem unusual. However, Price (1967) and later de Vlaming and Sage (1973) reported blood chloride levels higher than sodium, in other elasmobranch species. The present work was intended to examine the blood sodium picture more fully, especially with respect to salinity-change effects, and to compare these with previously obtained values for serum chloride.

MATERIALS AND METHODS

"Pyjama sharks" (Poroderma africanum (Gmelin)) were collected by SCUBA diving, from both the Atlantic and Indian Ocean sides of the Cape Peninsula, in water depths ranging from 5 - 20 m. All animals were kept in tanks of filtered, sterilized running sea water (13 ± 1°C) in the laboratory, as previously described (Haywood, 1973). Fish ranged between 0.5 and 0.8 m in length and 1 and 3 kg in weight. Both sexes were represented approximately equally.

A total of 20 animals were exposed to sea water of which the salinity was gradually altered, over the maximum tolerated range. In the laboratory, sea water was diluted with deionized water (buffered slightly alkaline with small amounts of Na_2HPO_4 and NaOH) and was concentrated by evaporation or (in the case of non-mixohaline solutions) direct salt addition. Sea water was replaced in part at regular intervals, in order to maintain pH at 7.9, as previously described (Haywood, 1973). Sodium and chloride concentrations of the sea water were checked regularly and after acclimation of fish at known values for 60-72 h (Haywood, 1973), blood samples were drawn from the sinus venosus of fish, for serum separation and analysis as previously described (Haywood, 1973). All changes in medium salinity were made slowly, in the order of 10-13 mM Na/l per day - after which the fish were then allowed the stated acclimation time before blood analysis.

In view of the blood sodium level being lower than that of chloride, for fish in normal sea water and diluted mixohaline solutions, it was decided briefly to examine serum potassium in such fish, since potassium is normally the next most abundant blood ion, after sodium and chloride. Blood potassium measurements were not made over the whole tolerated salinity range but merely in fish exposed to three different salinities, including normal sea water.

During exposure to different salinities, some fish were fed 0.25 - 0.5 kg teleost meat every 2 - 3 days, whilst others were fed similar amounts at different time intervals, up to once a month. Previous results have shown that serum chloride levels are not affected by how often fish are fed, within this time range, and it was therefore intended to check if this was also true for serum sodium levels.

Since results of further experimentation (not reported here) have suggested that sodium and chloride ions may be regulated independantly, fish were also exposed to a few non-mixohaline solutions, where the sodium or chloride level had been artificially adjusted so as to be different from the normal sodium/chloride ratio exhibited in normal sea water and mixohaline solutions. In this way, fish were exposed to solutions sometimes more dilute than sea water, but in which the sodium level was abnormally high, sometimes higher than the normal sea water level. Such solutions were usually prepared without greatly affecting pH, but the production of low-sodium solutions required dilution of medium with non-buffered water and resulted in a drop in pH, to about 6.4. Such a pH change cannot be overlooked and might itself produce an effect upon blood salt levels, although probably not to any great extent. However, in view of this, only one such solution was made up and used.

By keeping the volume of each blood sample taken below 1 ml, it was possible to use fish several times throughout the investigation, returning them periodically to stock tanks to recover and feed prior to re-use.

Analysis of chloride was carried out after Schales and Schales (1941) (accuracy ± 5 mM/l), sodium and potassium were measured by flame emission spectroscopy, using a Unicam SP 1900 Atomic Absorption Spectrophotometer. Over the concentration range of sodium values the accuracy was taken as ± 5 mM/l, but over the more dilute range accuracy was taken as ± 0.5 mM/l.

Table 9. Blood values from normally-fed fish in 3 approximately mixohaline media. (Mean ± S.D.)

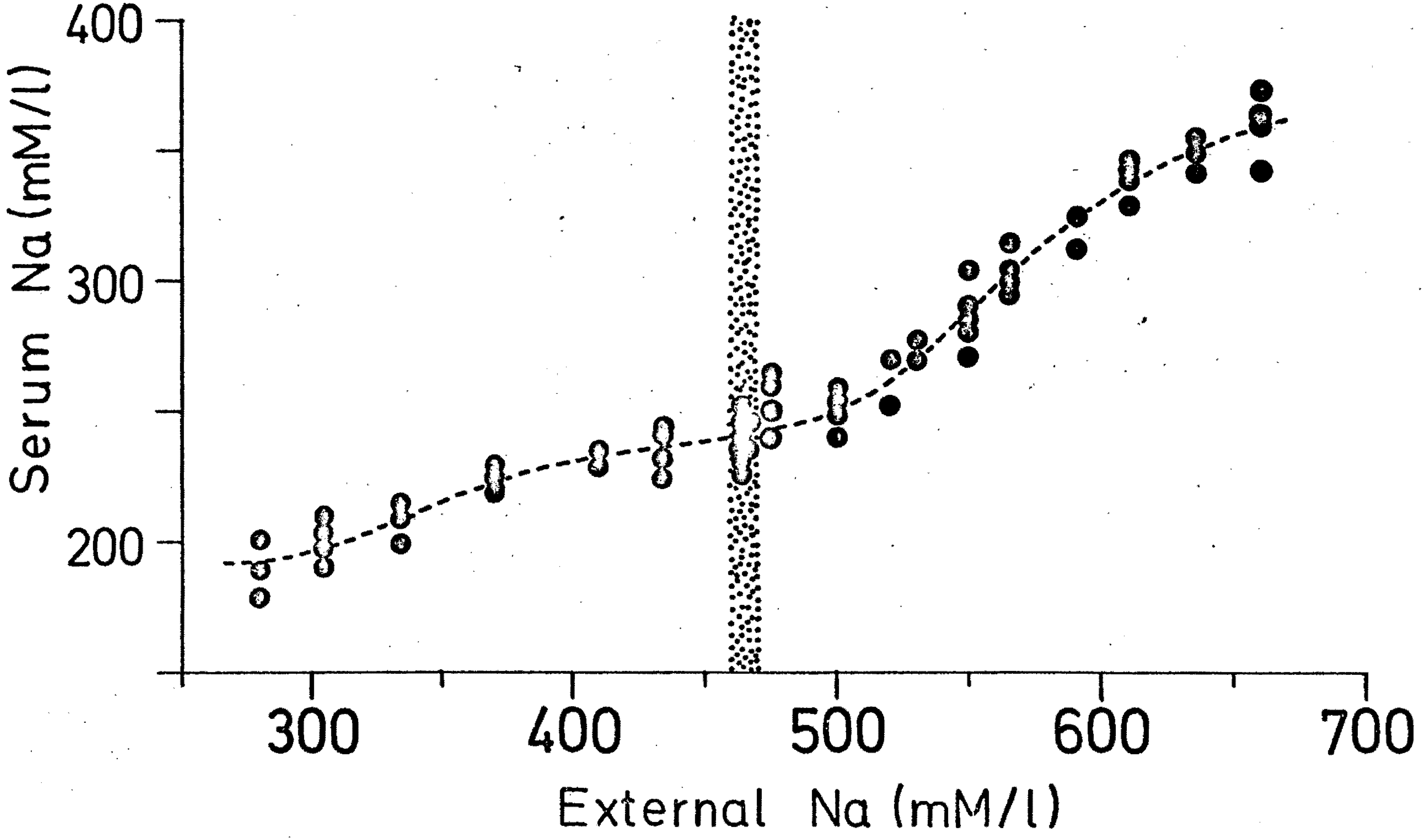
	1. Dilute medium		2. Normal Sea Water		3. Hyper-saline med.	
	S/W	Fish	S/W	Fish	S/W	Fish
Osmolarity mOsm/l	680	746 ± 20 (n = 5)	1030	1077 ± 10 (n = 5)	1180	1215 ± 11 (n = 4)
Cl mM/l	360	210 ± 4 (n = 5)	550	261 ± 5 (n = 8)	650	290 ± 3 (n = 4)
Na mM/l	305	201 ± 7 (n = 4)	467	238 ± 7 (n = 8)	565	302 ± 7 (n = 4)
K mM/l	6.3	3.0 ± 0.2 (n = 5)	9.6	3.2 ± 0.3 (n = 5)	11.1	4.7 ± 0.3 (n = 4)

RESULTS AND DISCUSSION

Fig. 10 shows the effect of change in external sodium concentration upon serum sodium, in P. africanum. The species was found to tolerate a range of external sodium from about 280 - 665 mM/1, under laboratory conditions. At these two extremities, serum sodium was approximately 190 and 365 mM/1 respectively. No difference in values was obtained between either males and females or between well-fed and under-fed fish, and Fig. 10 may therefore be taken as representative of all fish examined. It should be mentioned however, that salt-loading experiments and observations on fish which have ingested large quantities of invertebrate meat, such as cephalopod, do show a rise in blood salt level, over a period of a few hours after feeding, but this drops within about 7 hours. No such effect has been observed after ingestion of teleost meat.

Previous results (Haywood, 1974a), as included in Table 9, show that, in normal sea water, serum sodium averaged 238 mM/1, whilst serum chloride was 261 mM/1. Such results would seem unusual, since many marine fish exhibit higher blood sodium levels than chloride. Burger and Hess (1960) reported plasma salt values of 286 mM/1 for sodium and 246 mM/1 for chloride, in the spurdog, Squalus acanthias, although their figures quoted for the salt levels of the sea water used suggest a rather higher-than-normal sodium level. Later, Burger (1962) decided to measure chloride rather than sodium, when investigating salt levels in rectal gland fluid, because he decided that, in view of the lower chloride level, this would give a better minimal index of fluid salt level. Carrier and Evans (1972) also reported a higher blood sodium level than

FIGURE 10. Serum sodium concentration expressed against external sodium concentration. Stippled area denotes normal sea water range.



chloride, in the nurse shark Ginglymostoma cirratum, where blood sodium and chloride were 254 and 224 mM/1 respectively. Price (1967), however, reported that in the clearnose skate Raja eglanteria, blood sodium averaged 253 mM/1 whilst blood chloride was 271 mM/1, for fish in almost normal-strength sea water. More recently, de Vlaming and Sage (1973) have measured blood salt levels in the stingray Dasyatis sabina, exposed to different salinities, and have shown that plasma chloride can exceed plasma sodium by up to 23 mM/1 - a value which corresponds with present findings.

It might at first be assumed that salinity change would have a similar effect upon serum sodium and chloride, but in fact comparison of the present curve (Fig. 10) with that previously obtained for serum chloride (Haywood, 1973) shows that this is not the case. Whereas the effect of salinity change upon serum chloride was found to be reasonably even, over the majority of the tolerated salinity range, it can be seen that serum sodium rises quite sharply in media more concentrated than normal sea water. Such sharp rise, in media of above 500 mM/1 sodium (approximately 107% sea water), produces a change in the ionic composition of the blood. It can be seen from the discreet measurements of blood sodium and chloride, presented in Table 9, that, as the external salinity is raised, the difference between blood sodium and chloride values diminishes so that over the range of external sodium concentrations 530 - 550 mM/1 (113 - 117% sea water) serum sodium and chloride are about equal (approximately 270 - 285 mM/1). Further raising the salinity results in the serum sodium level exceeding that of chloride, so that, in the highest salinities tolerated, serum sodium is in the order of 350 mM/1, whereas serum chloride is only about 315 mM/1.

Table 10. Blood values (Mean ± S.D.) from normally-fed fish in 3 non-mixohaline solutions, artificially altered with respect to sodium.

	1. Low sodium		2. Raised sodium		3. High sodium	
	S/W	Fish	S/W	Fish	S/W	Fish
Osmolarity mOsm/1	850	867 ± 5	950	982 ± 13	890	919 ± 2
Cl mM/1	480	230 ± 2	505	246 ± 5	530	245 ± 6
Na mM/1	368	210 ± 2	430	248 ± 8	470	251 ± 3
	(n=5 all values)		(n=5 all values)		(n=4 all values)	

Both Price (1967) and Price and Creaser (1967) showed that transfer of skates into increased salinities produced only small increases in blood sodium chloride levels. Figures reported by Price (1967) suggest that sodium and chloride were altered about equally, but it should be remembered that their work was concerned with dilutions of the medium and no fish were exposed to medium above normal sea water salinity. In media below normal sea water, present results are similar.

Measurements of serum potassium, for fish in 3 approximately mixohaline media, showed that serum potassium is a little lower, even in normal sea water (9.6 mM K/l), than corresponding values reported for Squalus acanthias (Burger and Hess, 1960), where plasma potassium was reported as ranging from 4.4 mM/l to 7.0 mM/l. Price (1967) showed even higher serum potassium levels in the clearnose skate, where values were 7 - 13 mM/l. More recently, de Vlaming and Sage (1973) have reported a range of plasma potassium levels in the stingray Dasyatis sabina from 1.7 - 7.8 m-equiv/l, depending on external salinity. There is obviously therefore a degree of variation with species but present results show that serum potassium levels in P. africanum are by no means unusually high and do not form any sort of compensation for the rather low blood sodium levels observed in the more dilute media. Indeed, under such conditions, serum potassium is itself reduced.

Exposure of animals to 3 non-mixohaline solutions, (Table 10) in which the sodium level was either raised or lowered, with respect to chloride and osmolarity, shows that blood sodium correspondingly rises or falls. Consequently, animals exposed to medium which has a similar chloride level and osmolarity to normal sea water, but whose sodium level has been artificially raised above that of sea water, have been found to show a smaller difference in blood values between sodium and chloride and indeed the serum sodium level can exceed that of chloride if the external sodium concentration is raised sufficiently. Conversely, animals exposed to medium with artificially reduced sodium concentration showed blood sodium levels well below chloride. Since a solution artificially high in sodium may be also taken as being low in chloride (and osmolarity and other ions) each result could be viewed from either the sodium aspect or the chloride aspect and it therefore seems not unreasonable to suggest that the concentration of the major ions in the blood may well depend on the concentration of those same ions in the external medium. Thus each ion may well be regulated independantly, being directly affected by its concentration in the external sea water. Such an idea of ion-specific regulation is further supported in the case of P. africanum, by recent work (unpublished) with salt-loaded animals, in which the rectal gland appears to regulate sodium and chloride differently. De Vlaming and Sage (1973) have pointed out that "The decrease in concentration of solutes in stingray plasma is obviously not due to a simple dilution of the blood (i.e. increase in blood volume) if this were the case, all solutes would be diluted to an equal extent, and this was not observed. Since the percentage decrease in plasma solutes and ion ratios vary, individual regulation of solutes appears likely."

In conclusion therefore it seems that in P. africanum the effect of salinity change upon serum sodium and chloride levels is different - the difference being more apparent in hyper-saline media. In normal sea water and all mixohaline dilutions of it, serum sodium is lower than serum chloride. Exposure of animals to non-mixohaline solutions suggests that ions are regulated independently, but proportionally to their concentration in the external medium. Since local sea water often shows minor fluctuations in ionic and osmotic composition, depending on locality or season, fish taken from different locations at different times of the year may well show slightly different blood compositions.

CHAPTER 8

A PRELIMINARY INVESTIGATION INTO THE OSMOREGULATORY ROLES PLAYED BY THE RECTAL GLAND AND KIDNEYS.*

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ABSTRACT

A preliminary investigation was made into the ionic and osmotic regulation effected by the kidneys and rectal gland, in the striped dogfish _Poroderma africanum_, under laboratory conditions. Fish with inoperative glands showed only slight rise in blood sodium and chloride levels, as measured over a period of 2 weeks, and compared against control animals. Salt-loading of both fish with inoperative glands and control fish showed that blood salt levels returned to normal within 7 h. Comparison of the two groups indicated that the rectal gland may control chloride levels more so than sodium, although its effect was not found to be very pronounced in either case. Collection of a compound fluid, consisting of urine and rectal gland fluid, was made from normal fish, and the estimated cloacal salt loss is discussed. Urine was also collected separately, from normal fish, and was analysed for its contribution to salt loss. The various implications of present findings are discussed and compared with relevant results of other workers.

INTRODUCTION

In 1960, Burger and Hess formulated the hypothesis that the rectal gland in elasmobranchs, in particular that of *Squalus acanthias*, acts as a salt gland, capable of aiding the ionic and osmotic balance between internal and external medium, by the secretion of a fluid "containing sodium chloride at about twice the plasma concentration and at a concentration higher than that of the external sea water". They further pointed out, from data obtained, that, "through the combined action of the rectal gland and kidneys, the dogfish is able to eliminate sodium chloride at roughly corrected sea water concentrations". Since the urine produced was found to be hypotonic, with respect to the blood, this regulation was not considered possible by the kidneys alone.

Later work by Burger (1962, 1965) further supported this earlier suggestion and it was shown (Burger, 1962) that from 5-32% of the plasma NaCl passing through the gland could be extracted. Moreover, the average glandular NaCl secretion was twice the urinary secretion, although the glandular secretion rate was variable - from 0-1.9 ml/h/kg body weight. Rectal gland secretion in the stingray *Dasyatis sabina* was also examined (Burger, 1972) but only with respect to chloride and secretion rate. Burger has reported the chloride concentration as being nearly twice that for *Squalus acanthias*, although the rate of chloride secretion in the two elasmobranchs is similar.

More recent work, however, has suggested that the rectal gland may not be as important in salt output, as was thought by Burger. Maetz and Lahlou (1966) showed that, in the dogfishes *Schyliorhinus* *canicula* and *S. stellare*, both the urinary and rectal gland salt outputs were minimal, by comparison with branchial output. Burger and Tosteson (1966) showed that the gills of *Squalus acanthias* may be an important source of ion transfer, and later, Horrowicz and Burger (1968) confirmed this finding, when they reported a branchial ion flux rate ten times greater than that measured through the skin. Payan and Maetz (1970) found that branchial salt regulation in the genus *Scyliorhinus* accounted for two-thirds of the total sodium efflux. By comparison, rectal gland secretions were minimal, providing only one-tenth of the total sodium efflux. Payan *et al.* (1973) have shown that branchial urea output is far in excess of renal output, and it seems that the role of the rectal gland may have been overemphasized in the past, whereas the role played by the gills has not been fully appreciated. Previous work (Haywood, 1974b) has indicated that branchial salt regulation may well be important in *Poroderma africanum*, and that the gills and kidneys between them may handle the majority of salt output. In view of these findings, the present investigation was undertaken to examine the roles of the kidneys and rectal gland, in more detail.

MATERIALS AND METHODS

ANIMALS

Specimens of *Poroderma africanum* (Gmelin) were collected from both sides of the Cape Peninsula and kept in the laboratory as previously described (Haywood, 1973). Fish ranged between 0.2 and 0.8 m in length and 0.3 and 3.0 kg in weight, for this series of experiments. Both sexes were represented approximately equally. Laboratory temperature was maintained at $13 \pm 1^{\circ}C$ and fish were fed teleost meat and small whole cephalopods at regular intervals, until 48 h prior to experimentation.

INOPERATIVE RECTAL GLANDS

Four experimental animals were anaesthetised in sea water containing 0.03% MS 222 (tricainemethanesulphonate) as previously described (Haywood, 1974a) and the duct joining the rectal gland to the gut was surgically tied off (after Haywood, 1974a) so that no secretion from the rectal gland was possible. After suturing the body cavity, fish were allowed to recover in specially darkened tanks, since is has been found that animals kept in the dark remain inactive with resultant little stress on new sutures. Blood samples were taken (after Haywood, 1974a) at regular intervals over a period of 2 weeks, as indicated in Table 11. Two control fish, with untouched glands, were also used for comparison. The composition of sea water used is presented in Table 11.

SALT LOADING

In a separate experiment, 5 fish, each approximately 1 kg in weight, had their rectal glands surgically tied, as previously described, and were then allowed to recover in shaded tanks. Four unoperated animals were kept under similar conditions. After 24 h, both experimental and control animals were each injected with 2 ml of 750 mM/l sterile NaCl solution into the peritoneal cavity. Previous results had indicated that a rise in blood NaCl concentration is apparent within ½ - 3 h after such injections. Blood samples were drawn from all fish at time intervals 0 (immediately after injection), ½, 1, 2, 3 and 7 h after injection and again after 1, 2 and 3 days. The composition of the sea water is shown in Table 12.

CATHETERIZATION

During this research it was intended to collect urine and rectal gland fluid separately from fish, by means of in-dwelling catheters. However, this technique was not successful and attempts had to be discontinued.

COMPOSITE FLUID

An experimental arrangement was designed to collect urine and rectal gland fluid together at the cloaca. Small fish (approximately 0.5 kg weight) were used for this work.

Each fish was anaesthetised in sea water containing 0.03% MS-222 to facilitate the following procedure. A hole, of approximately 2 cm diameter, was cut in a piece of thin rubber sheeting. When completely anaesthetised, the fish was removed from the sea water and quickly dried with tissue. The hole in the rubber sheet was stretched and the head of the fish inserted until the pectoral fins had just passed through. The rubber was then allowed to return to normal dimensions, thereby gripping the fish just posterior to the pectoral fins and forming a seal around the body. The anterior portion of the fish was placed inside a polythene tube, closed at one end, but with water inlet and outlet pipes in the closed end. The rubber sheeting was secured over the lip of the open end of the tube so that the anterior half of the fish was then enclosed in a sealed container, through which running sea water could be passed. The exposed part of the fish was then thoroughly dried, especially in the region of the cloaca, and any faecal material in the rectum was carefully removed. Whilst under anaesthetic, an absorbent gauze plug was inserted into the rectum and pushed as far down (anteriorly) as possible. This was intended to prevent defaecation during the experiment. Fish for this particular experiment were not fed for 1 week prior to this stage. Aerated sea water of normal salinity (not drugged) was then recycled through the sealed tube and the posterior region of the fish, after ensuring complete dryness, was immersed in a shallow container of liquid paraffin. With practice, all procedures up to this stage were achieved in less than 1 minute, after removal from drugged sea water.

The container of liquid paraffin was tilted slightly, and, since aqueous fluid and liquid paraffin are immiscible, with the aqueous solution forming the lower phase, any droplets of aqueous fluid released from the cloaca eventually collected in the lower corner of the container, from where they could be removed, still under paraffin, by a specially designed capillary-tube pipette. Faecal material is produced as a thick mucoid jelly in Poroderma africanum and can be easily distinguished from cloacal fluid. On only one occasion was faecal material observed in the collecting dish, and the fluid was therefore discarded in this case.

By circulating drug-free sea water through the anterior sealed capsule, it was possible to return fish to consciousness within a few minutes. Although this necessitated restraining fish somewhat, it was considered better to work with undrugged animals, since the drug would eventually be excreted in the urine, with resultant effect on the osmolarity of the fluid collected. In all cases, fluid was not collected for a period of ½ h after fish regained consciousness, in order to reduce the possibility of contamination of collected fluid by excreted drug. Depending on the rate of fluid production, animals were maintained in the collecting apparatus for up to 24 h, during whichtime they were kept in near-total darkness, to reduce fretting.

The volume and secretion rate of collected fluid was recorded, together with its compostion.

URINE

It was found possible to collect urine separately from large animals (over 2.5 kg weight) by removing them from the water, drying the cloacal region and urogenital papilla thoroughly and then gently squeezing the abdomen several times. In most cases this stimulated the production of a brief jet of urine from the papilla, which could be collected in a clean dry glass container, strategically positioned. Animals were not drugged for this procedure. Since a more distinct papilla is present in the male, making collection easier, all urine collected from the present assay was obtained from 6 male individuals, exposed to normal sea water.

FLUID CONCENTRATIONS

The osmolarity of all fluids assayed was measured by freezing-point depression, using a Knauer osmometer (accuracy ± 5 mOsm/l). Chloride was measured after Schales and Schales (1941) (accuracy ± 5 mM/l) and urea by the method of Pré et al. (1968) (accuracy ± 1%). Sodium was assayed with a Unicam SP 1900 Atomic Absorption Spectrophotometer (accuracy ± 5 mM/l).

RESULTS

The effects upon blood composition, due to ligation of the rectal glands of experimental fish, are compared with those of control animals, over a period of 2 weeks, as presented in Table 11.

Table 12 presents the effects upon blood values after salt loading both control and experimental fish with inoperative rectal glands.

The average composition and secretion rate of the composite fluid collected from the cloaca of 5 animals exposed to normal sea water is presented in Table 13.

Table 14 presents the average composition of urine collected from 6 animals exposed to normal sea water.

Table 11. Blood values in control and experimental fish with inoperative rectal glands, over a period of 2 weeks. Om = osmolarity (mOsm/l) Na and Cl expressed as mM/l Fish 3 & 4=controls.

	Fish 1			Fish 2			Fish 3			Fish 4			Fish 5			Fish 6		
Days	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl
0	960	232	240	955	230	245	960	242	255	960	245	258	1065	261	280	1050	260	280
1																		
2				960	258	265							1065	265	304	1050	261	288
3	960	256	260															
4				1000	252	286	960	244	255	960	245	257	1065	266	312	1060	265	312
5	965	260	270															
6													1065	278	312	1060	269	296
7				960	240	250	960	242	257	965	245	255						
8	960	255	250										1070	276	310	1055	267	298
9																		
10																		
11	960	254	245				960	242	257	960	247	257						
12																		
13																		
14				955	238	252	960	242	255	960	245	257	1075	275	300	1050	263	290
15	960	237	255															

	Om	Na	Cl
Sea water	940	440	535

	Om	Na	Cl
Sea water	1030	470	570

Table 12. Blood values in control and experimental animals after salt loading. Om = osmolarity (mOsm/l) Na and Cl expressed as mM/l. Fish 3, 4, 7 and 8 are controls with normally-functioning rectal glands. 0 = time 0. Final measurements 3 days after salt loading.

	Fish 1			Fish 2			Fish 3			Fish 4			Fish 5			Fish 6			Fish 7			Fish 8		
Time	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl	Om	Na	Cl
0	990	235	260	100	242	265	990	240	256	990	235	265	1000	252	270	1000	263	280	1020	262	272	1020	278	272
½ h	1010	270	320	1000	242	277	1000	245	256	1000	252	276	1005	259	296	1010	265	292	1020	262	272	1020	280	272
1h	1000	263	280	1010	255	290	1020	313	270	1020	271	288	1010	270	300	1020	283	296	1030	272	272	1040	286	272
2h	990	252	264	1020	270	295	1015	282	265	1000	256	272	1020	283	310	1025	286	296	1050	292	280	1045	290	272
3h	1000	261	260	1010	255	280	1010	263	262	995	240	272	1010	256	304	1005	265	288	1025	296	275	1030	280	275
7h	1000	261	264	1010	250	275	1000	265	260	990	236	272	1000	248	272	1000	263	285	1010	265	265	1020	278	275
1d	1010	260	280	1010	245	270	1000	261	260	990	235	275	1000	256	270	1000	265	282	1020	265	270	1020	272	272
2d	1010	274	285	1010	248	270	995	243	250	990	236	272	1000	248	272	1000	265	285	1020	268	275	1020	275	272
3d	1020	287	296	1020	250	275	990	242	256	990	236	272	1005	263	272	1005	270	288	1020	265	272	1020	278	272

	Om	Na	Cl		Om	Na	Cl
Sea water	960	465	555	Sea water	990	492	573

Table 13. Average values for composite fluid collected from the cloaca of *Poroderma africanum* in normal sea water (Om 1020, Na 467 Cl 550mM/1) For all values n = 5.

Sodium mM/1	Chloride mM/1	Urea mM/1	Secretion rate ml/kg/day
403 ± 7	438 ± 6	136 ± 23	3.0 ± 0.3

Table 14. Average composition of urine collected from Poroderma africanum in normal sea water. (Om 1020, Na 467 Cl 550 mM/l) For all values n = 6.

Osmolarity mOsm/l	Sodium mM/l	Chloride mM/l	Urea mM/l
935 ± 14	185 ± 24	249 ± 22	176 ± 32

DISCUSSION

Results in Table 11 indicate that experimental fish with inoperative rectal glands experienced initial rise in blood sodium and chloride levels which stopped after about 1 week and, in most cases, began to fall again shortly afterwards. Control animals showed constant blood values over the experimental period, so that comparison of the two groups indicates that inactivation of the rectal gland produces a short-term response which is minimal and which seems to be compensated by regulation elsewhere within a short time period. Under such conditions, blood chloride was found to rise a little more than sodium although further work is required to show any significant difference between the two.

Salt loading of fish produced rises in blood sodium and chloride levels in both control and experimental animals. Comparison of the two groups, however, suggests that in control animals, with normally functioning rectal glands, rise in blood chloride was less than rise in sodium. The converse was apparent in experimental animals with non-functioning rectal glands. In both cases rise in blood salt levels was apparent within ½ - 3 h after injection of sodium chloride. In both control and experimental animals blood values began to fall again after the initial rise although experimental animals would be expected to show slightly raised blood values after 3 days, due to inactivated rectal glands, even after the effect of salt loading had passed (as shown by fish in Table 11). Such findings indicate that the rectal gland may not regulate chloride and sodium to the same extent although its overall effect upon blood sodium and chloride levels seems minor.

Burger and Hess (1960) suggested that the rectal gland acts as a salt gland but later (Burger, 1962, 1965), it was shown that dogfish with rectal glands rendered non-functional were able to stabilise plasma chloride for up to 8 days, although the total excretion of chloride was less than in fish with functional rectal glands. Burger also showed that injection of sodium chloride into dogfish produced a characteristic response, with a lag of ½ - 1¼ h before secretion from the gland reached a maximum. The rate and duration of secretion was found to be roughly proportional to the amount injected. Burger (1965) pointed out that "the rectal gland is a specific regulator of sodium chloride and uniformly concentrates salt". He also stated, however, that "in the absence of the rectal gland, a plasma composition comparable to that of the intact fish can be maintained both in full and dilute sea water". This he attributed to a decrease in the uptake of salts by the fish. He reported that 4 - 5 h after injection of NaCl, plasma chloride rose by about 20 - 30 mM/l, with only a rise in osmolarity of 10 - 15 mOsm/l. Similar findings are reported for the present investigation but unfortunately Burger did not measure plasma sodium after salt loading and in view of this, his conclusion that the rectal gland "uniformly concentrates salt" does not seem justifiable.

Chan _et_ _al_. (1967) showed that in lip-sharks (_Hemiscyllium plagiosum_) receiving a hypertonic saline load, there was no change in plasma sodium concentration but there was a marked rise in muscle Na content. They were unable to decide whether this was the result of influx of Na into the cells or due to a mere expansion of the extracellular spaces. In sharks from which the rectal glands had been removed, force-feeding resulted in a slight but significant rise in plasma Na concentration, whilst the rise in muscle Na content was considerable. They found that saline injection increased rectal gland secretion only temporarily, and they stated that all three organs, the gills, the rectal gland and the kidneys, seem to contribute jointly to excrete excess salt in the face of a salt load.

Present results indicate that the composite fluid from the cloaca is produced at a very low secretion rate, averaging 3.0 ml/kg/day. Unfortunately, by the nature of the collection technique used, it is not possible to anticipate what fraction of this fluid is due to urine and to rectal gland fluid. Burger and Hess (1960) have given a maximal output rate for combined rectal gland fluid and urine, in Squalus, as 169 ml over 12 h for a 6.4 kg fish - which indicates a secretion rate of 52.8 ml/kg/day. It should, however, be born in mind that, whilst this is far in excess of present findings, their results indicate that the blood of Squalus acanthias is maintained more hyper-osmotic, with regard to the external medium, than is usual for Poroderma africanum. Consequently the osmotic water influs will be proportionally different, with correspondingly different fluid output. Potts and Parry (1963) have reported an average urine output of 5 - 20 ml/kg/day for elasmobranchs but even assuming the composite fluid to be entirely urine, this would only give a urine production of 3 ml/kg/day for Poroderma africanum. This may be explained by previous findings (Haywood, 1973) which show that this species maintains blood osmolarity only 40 - 60 mOsm/l above the external medium, in most salinities. It has also been shown that water exchange occurs at the gills (Haywood, 1974b) and therefore the very low cloacal fluid production might well suffice for osmotic equilibrium in this species.

Analysis of the composite fluid and relation of this to secretion rate indicates a cloacal salt loss of 5.03 µM Na/100g/h, 5.48 µM Cl/100g/h and 1.70 µM urea/100g/h. It should be noted that the possibility of faecal contamination of the composite fluid does exist, although precautions were taken to minimise this. Such contamination would probably increase the apparent salt concentration of the fluid slightly so that the values obtained, if affected by contamination, will be slightly higher than the true values. In view of this, the calculated salt loss via the cloaca may be taken as a maximum value and it seems most unlikely that salt loss will be in excess of the calculated values.

By referring to previous overall turnover values for Poroderma africanum (Haywood, 1974a), the above calculated values indicate that the cloacal salt loss accounts for only 27.2% total sodium turnover, 14.2% total urea turnover and only 2.6% total chloride turnover. Thus over 97% of the chloride loss from fish seems to occur at some other site - most probably the gills and/or epithelial surface. Such findings are consistant with those of several previous workers. Maetz and Lahlou (1966) showed that, in Scyliorhinus canicula and S. stellare, both urinary and rectal gland salt outputs were minimal by comparison with that of the gills. Burger and Tosteson (1966) have also shown that the gills of the spurdog, Squalus acanthias are sites of salt transfer - findings later confirmed by Horrowicz and Burger (1968). Payan and Maetz (1970) have shown that, in the genus Scyliorhinus, two-thirds of the total sodium efflux occurs at the gills, whereas only one-tenth is effected by the rectal gland. Boylan (1967) reports a branchial urea exchange of about 25 µM/100g/h in Squalus acanthias - which is in excess of the total urea turnover measured for _Poroderma africanum_ (Haywood, 1974a). It seems well established that branchial urea loss is greater than cloacal urea loss in most marine elasmobranchs and present results support this, since nearly 86% of total urea loss for _Poroderma africanum_ must be accounted for elsewhere but via urine and rectal gland fluid. Payan _et al_. (1973) have reported branchial urea outputs in the skates _Raja erinacea_ and _R. radiata_ which were far in excess of renal outputs - the latter being responsible for less than 10% urea loss.

By comparison with serum values for _Poroderma africanum_ in normal sea water (Haywood, 1974a), the present urine values show that the species produces a markedly hypo-osmotic urine (by over 130 mOsm/l) and that the urine is also hypotonic with respect to sodium and urea. The deficit is only slight for chloride, however, so that the urine is almost isotonic to the blood, with respect to this ion.

Whilst no assessment of urine production rate was possible in the present investigation, it is possible to estimate salt loss via urine, if the total cloacal fluid is assumed to be urine. It does not seem unreasonable that a large proportion might well be urine - especially if the average urination rate given by Potts and Parry (1963) is considered. Assuming a urine production of 3.0 ml/kg/day, the respective losses of chloride, sodium and urea would be 3.1, 2.3 and 2.2 μM/100g/h. This indicates a greater urea loss than previously shown for the composite fluid but rectal gland fluid has been shown to contain very little urea (Burger and Hess 1960) and therefore any rectal gland fluid present would apparently lower the urea concentration of the overall fluid. The converse is also true in the cases of sodium and chloride, which are more concentrated in rectal gland fluid than in urine. Consequently, the composite fluid collected at the cloaca represents urine and rectal gland fluid combined.

Whilst it is appreciated that further experimentation of a similar nature is required before any definite conclusions can be drawn regarding the roles of the rectal gland and kidneys in the osmoregulation of this species, certain facts have emerged which tend to support previous findings of other workers. Namely, the rectal gland does not seem to be as important a regulator as was originally thought. Its action in the face of salt loading seems to be minimal and only apparent over a short time period. The combined salt output through urine and rectal gland fluid seems to represent only a minor fraction of the overall loss, so that other organs such as the gills must be much more efficient regulators. Finally, and a point which does not seem to have been considered before, the rectal gland may not regulate chloride and sodium - indeed any salts - to the same degree. The general regulation of sodium and chloride in this species has been previously shown to differ (Haywood, 1974c) and fish seemed to regulate each ion independantly of other ions, but proportionally to the external concentration of the ion in question. It does not seem unreasonable to suggest that a similar independant regulation of ions occurs in the rectal gland of this species.

CHAPTER 9

A PRELIMINARY HISTOCHEMICAL EXAMINATION OF THE RECTAL GLAND.*

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INTRODUCTION

The anatomy of the rectal gland of elasmobranchs has been previously examined at the light microscope level by Sullivan (1908), Hoskins (1917) and Crofts (1925), and at the electron microscope level by Doyle (1962). In most elasmobranchs, the rectal gland is a compound club-shaped gland, opening into the dorsal surface of the rectum, immediately anterior to the cloaca, in the region of the pelvic girdle. Previous examination, by the above workers, has shown the gland to be composed of 3 layers; an outer fibromuscular layer with small arteries, a middle glandular layer, consisting of tubules and capillaries, and a central region, consisting of ducts and veins arranged around a central canal that terminates in the drainage duct to the rectum.

Bonting (1966) has examined the rectal glands in several elasmobranch species and reports that in the spiny dogfish, sand shark, smooth dogfish and sickle-shaped shark the tubules are arranged radially away from the central canal, while in the butterfly ray, sting ray, eagle ray and monkfish, the glandular parenchyma is divided by thin fibrous tissue septa into lobules, with tubules orientated mostly in a longitudinal direction. The tubules in the first group run into ducts which form part of the central canal, but are separated by capillaries.

It was not till 1960 that Burger and Hess suggested a function for this organ in the elasmobranchs. They showed that the rectal gland in the spiny dogfish Squalus acanthias acted as a salt gland, producing a concentrated fluid composed almost entirely of sodium chloride. In so doing, they suggested, the rectal gland helps to maintain the ionic balance in the fish, compensating for the passive influx of ions together with salt ingested with food. Later work (Burger, 1962, 1965) further supported this suggestion and emphasized the role of the rectal gland as an ionic regulator. Burger (1962) showed that ligation of the gland resulted in a rise in both plasma and urine chloride levels, though no measurements of blood or urine sodium levels were made. Salt loading of animals was also shown to increase rectal gland secretion rate. Further evidence supporting the suggestion that the elasmobranch rectal gland is a salt gland was provided by Oguri (1964) who compared rectal glands in fresh-water and marine elasmobranchs. He reported that "a remarkable difference was observed in the size of rectal glands from marine bull sharks (*Carcharhinus leucas*) compared to those from Lake Nicaragua and Rio San Juan, even if the smaller size of the fresh-water sharks is taken into consideration".

Recent findings for *Poroderma africanum* (Haywood, 1974d) have indicated that the rectal gland (a) is not as important a regulator of body ionic concentration as was previously thought and (b) may not control blood chloride and sodium to the same extent. Results tended to suggest greater regulation of chloride than sodium. In view of previous results (Haywood, 1974b) which suggest that 'Chloride Cells' are present in branchial tissue of this species, and the above findings, it was decided to extend the chloride test to rectal gland tissue, at the light-microscope level.

MATERIALS AND METHODS

ANIMALS

Specimens of Poroderma africanum (Gmelin) were collected from both sides of the Cape Peninsula and kept in the laboratory, as previously described (Haywood, 1973). Both sexes were present in approximately equal numbers. The investigation was carried out on 8 fish, acclimated to normal sea water (467 mM Na/1, 550 mM Cl/1, 1030 mOsm/1) for a period of 1 week, and fed up to 48 h before experimentation.

TISSUE PREPARATION

The rectal glands from 4 normal animals were compared with those from 4 fish which had been salt-loaded. Animals were removed from sea water, weighed and then stunned with a sharp blow to the dorsal cranial surface. The rectal gland was quickly removed through a mid-line incision in the pelvic region. The length and greatest width of the gland were measured, together with its fresh weight (values not reported here). Rectal gland tissue for the chloride test was then quickly transfered to deionized water at 5°C, for 15 minutes, to remove surface salts, after being cut longitudinally or transversely for later sectioning. Tissue for normal histological staining was transferred to 2.5% formal saline and stored overnight at 5°C.

Four animals were injected inter-peritoneally with 2 ml (fish under 1.5 kg) and 4 ml (fish over 1.5 kg) of a 750 mMolar NaCl solution. Two hours after injection, fish were removed from the sea water and the rectal glands excised as described above. Such fish were considered to be salt loaded and previous results (Haywood, 1974d) indicate that the rectal gland should be most active from ½ -3 h after salt injection.

The chloride test staining procedure was that of Copeland (1948) as modified by Haywood (1974b).

Tissue for normal histological staining was dehydrated through the various stages of alcohol and both this tissue and that exposed to the chloride test were then cast into wax blocks (M.P. 52°C) for sectioning. Sections were cut at 5 - 10 μ.

Sections for PAS staining were dewaxed in xylene and run through descending strengths of alcohol, to distilled water. They were then oxidized for 5 minutes in 0.5% aqueos periodic acid, washed in distilled water and stained in Schiff's reagent for 10 minutes. After staining, tissue was rinsed in a metabisulphite rinse (sodium metabisulphite 8g, HCl Conc. 20 ml, Dist. H_2O 2 litres) for 2 minutes and washed in running tap water for 5 minutes. Further staining in eosin haematoxylin was carried out for 2 minutes. After further washing, tissue was differentiated in acid alcohol, lightly blued in ammonium hydroxide, rinsed and then dehydrated and mounted in the normal manner.

Tissue was also stained with the following composite stains, Alcain blue-Neutral red, Mallory's stain-Sudan IV, and Phosphomolybdic acid orange-Sudan black.

Tissue preparations were photographed with a Zeiss Photomicroscope II 1540 on Ilford Pan F film and the negative developed with a Beutler developer (K. Oates - personal communication), to increase tonal range.

RESULTS

HISTOLOGY

The general arrangement of tubules in the rectal gland of Poroderma africanum was shown with Phosphomolybdic acid orange-Sudan black staining. The appearance of the gland in T.S. is shown in Plate 12 and, with increased magnification, tubules and individual cells were visible (Plate 13) although not to the degree of resolution desired. Many tubules appear to be running almost parallel to the long axis of the gland, although the majority lie in a radial arrangement away from the central collecting duct. As later indicated, the arrangement of tubules was not found to be uniform throughout the length of the glands examined.

PAS staining revealed an overall radial arrangement of tubules (Plate 14) in the upper region of the gland, but several tubules were again found to be running at irregular angles to the majority. Some tubules produced the appearance of spaces or subsidiary collecting ducts running parallel to the long axis, possibly linking up with the central duct in the lower region of the gland. Blood vessels (mainly veins) were abundant in the central regions of the gland. Fibrous material in the outer fibro-muscular layer was heavily stained and the tubules and capillaries in the middle glandular layer also showed positive staining. The passage of collecting tubules into the central canal is shown in the L.S. preparation of the upper region of a gland (Plate 15).

Results with Mallory's stain-Sudan IV showed similar arrangement and definition to that with Phosphomolybdic acid orange-Sudan black

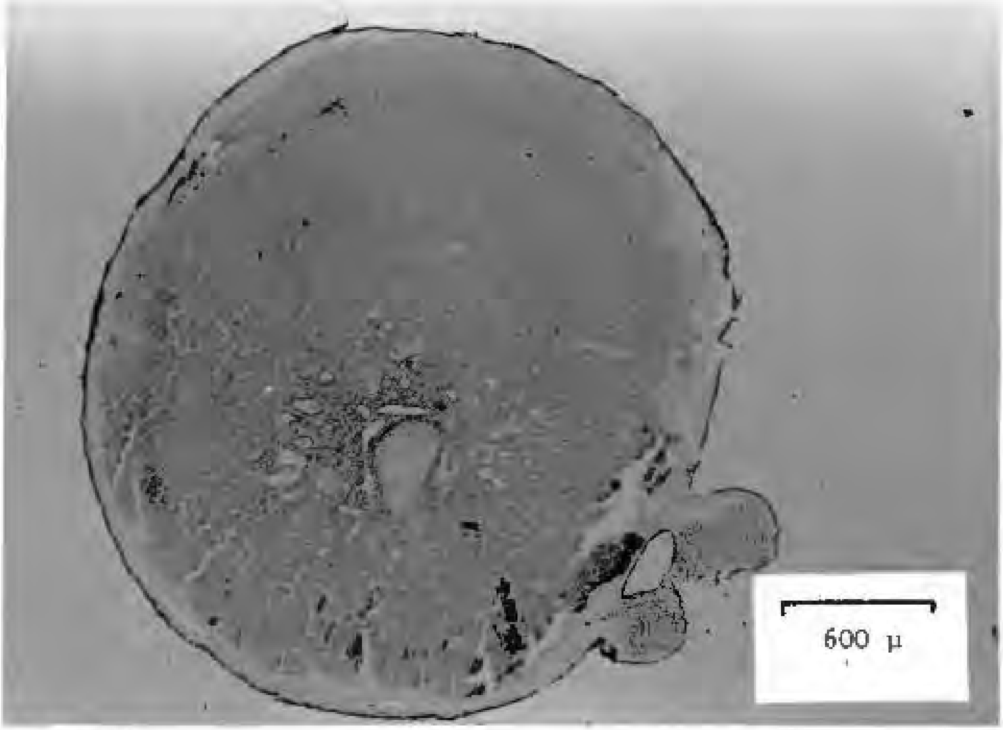

PLATE 12.

PLATE 13.

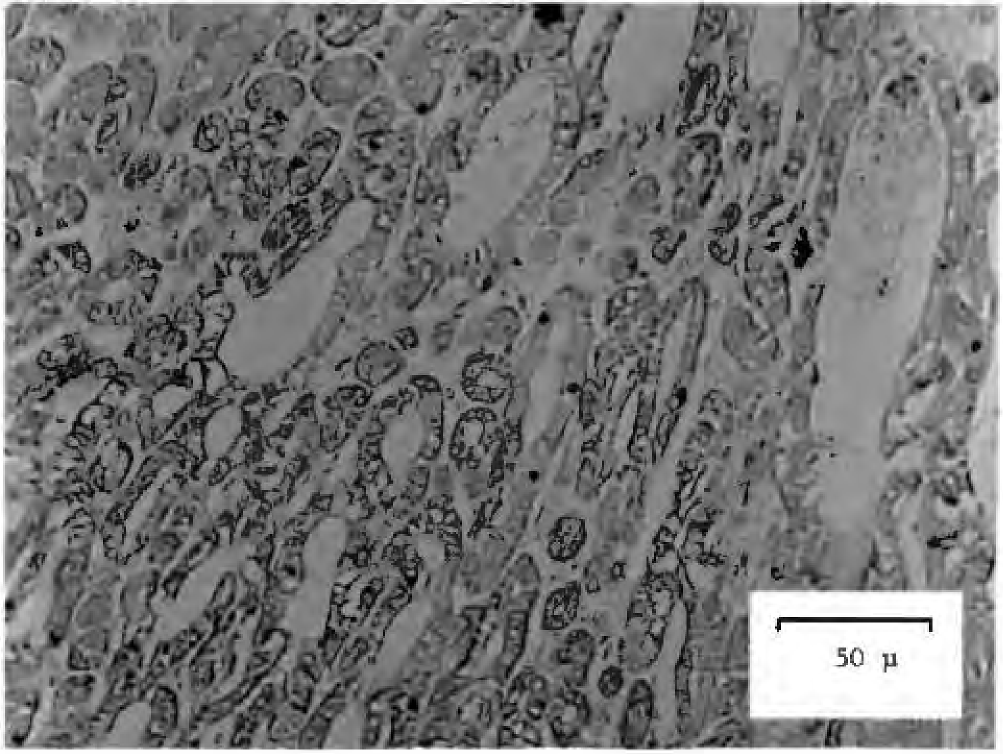

PLATE 12. Transverse section through the upper region of a small rectal gland, stained with Phosphomolybdic acid orange-Sudan black.

PLATE 13. Ducts and tubule cells under higher magnification, as shown with Phosphomolybdic acid orange-Sudan black staining.

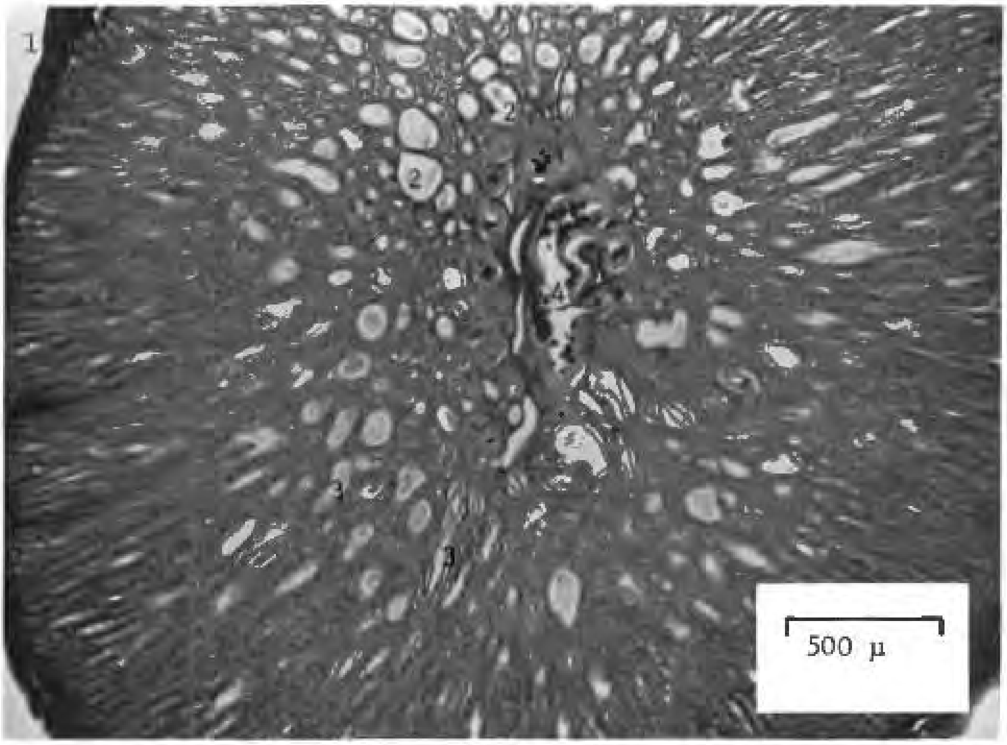

PLATE 14.

PLATE 15.

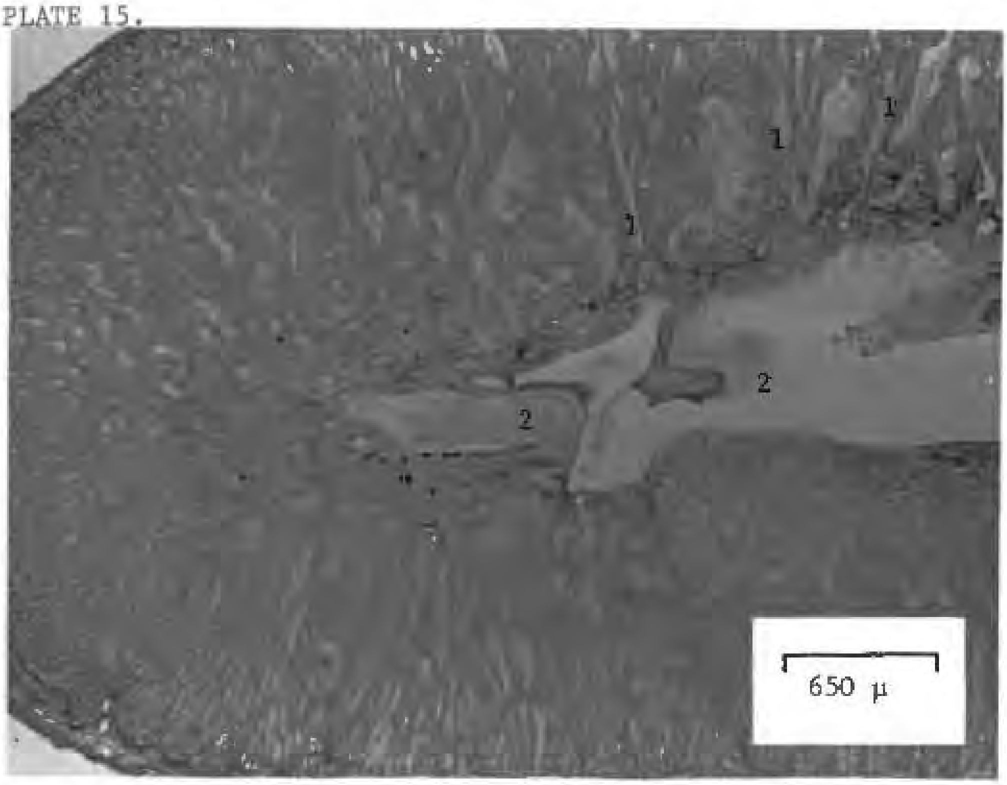

PLATE 14. Transverse section through the upper region of a PAS-treated gland, showing the predominant radial arrangement of tubules away from the central canal. Note (1) PAS-positive fibrous outer layer; (2) veins; (3) tubules at oblique angles to the usual radial orientations; (4) central canal with PAS-positive mucinous material.

PLATE 15. Longitudinal section through PAS-treated gland. General radial arrangement of tubules is apparent in the upper region of the gland. Note (1) ducts opening into central canal; (2) mucinous material in lumen of canal.

but results with Alcian blue-neutral red were unsatisfactory.

CHLORIDE TEST

Silver staining, followed by gold toning, for the chloride test, revealed a high concentration of silver granules deposited in tubule cells and ducts of the central region of the gland, even in normal fish (Plate 16). Although these appeared to be concentrated in the more central region they were apparent out as far as the middle glandular region and seemed to form a ring of silver granules over the mid-region of the gland, as seen in T.S. (Plate 17). Similar treatment of tissue from salt-loaded fish showed high densities of silver deposit in the ducts and tubules leading to the central canal. This is shown in Plate 18, where the radial arrangement of tubules in the upper region of the gland is still apparent, and also in Plate 19, in the lower region of the gland, where the tubules appear to be divided up into discrete lobules by septa of a fibrous (PAS positive) nature. Again silver granules were apparent as far out as the middle glandular regions. Closer examination of tissue from normal, but especially from salt-loaded fish, shows the silver granules to be intracellular. In some cases silver deposits were apparent within the lumen of tubules suggesting silver deposits due to chloride from rectal gland fluid, but in most cases the silver granules appeared to lie within the cytoplasm of the tubule cells (Plates 20 and 21).

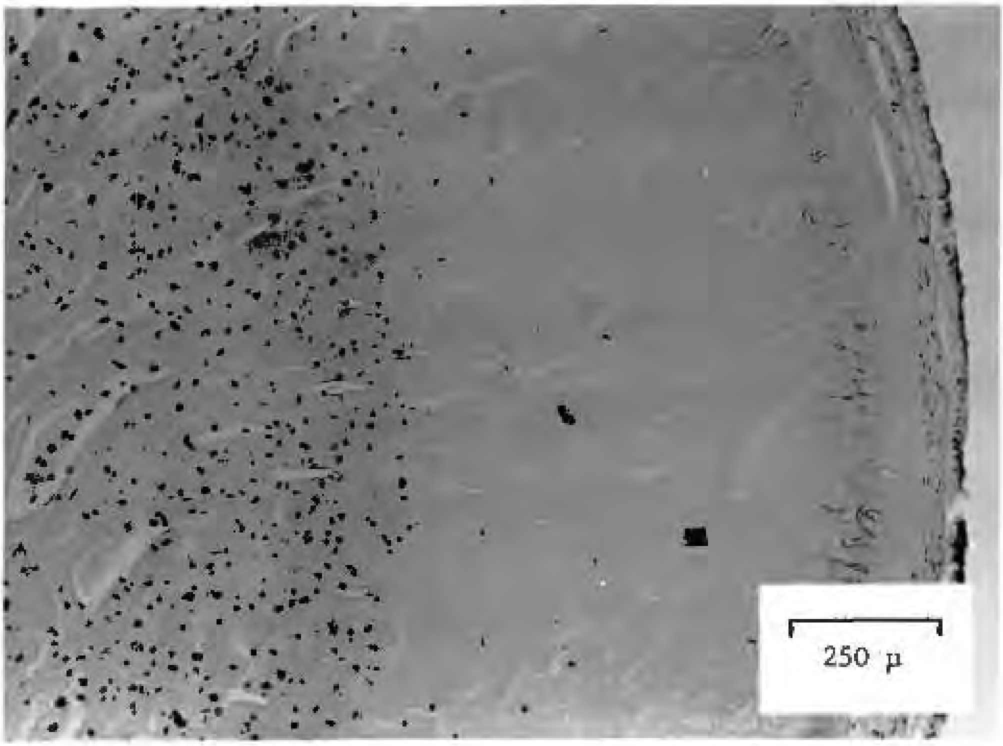

PLATE 16.

PLATE 17.

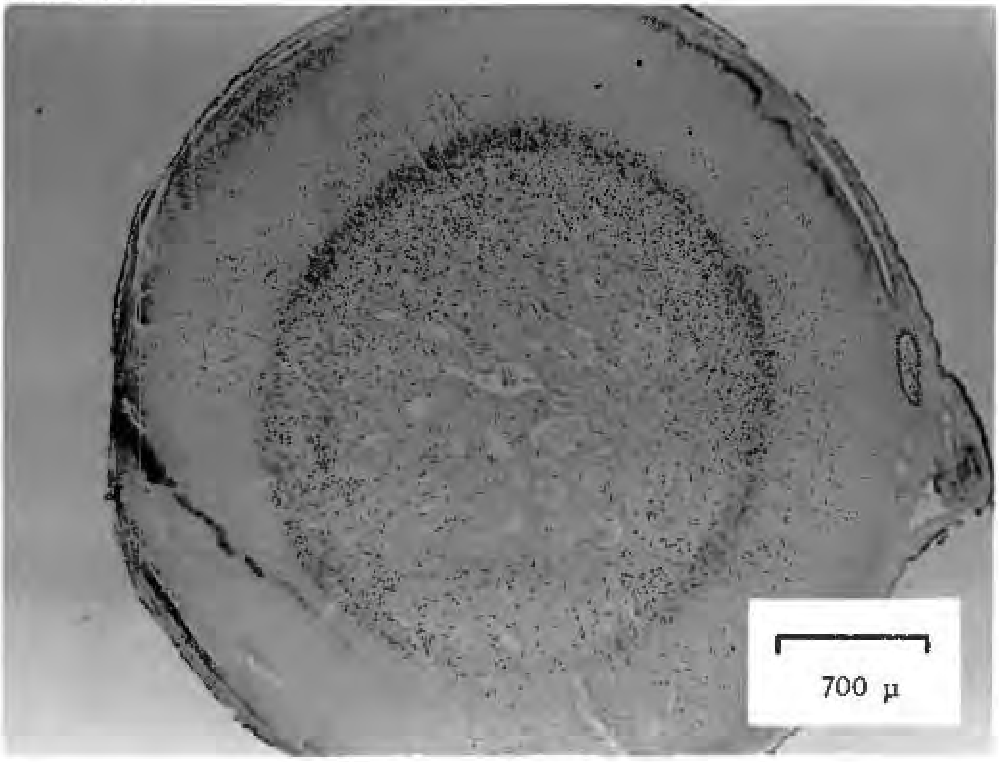

PLATE 16. Transverse section of rectal gland, after silver staining, showing the distribution of silver granules in tubule cells of the more central regions of the gland, extending out to the inner regions of the middle glandular layer.

PLATE 17. The general distribution of silver granules, from the inner regions out to the middle glandular layer, is illustrated in this low power transverse section.

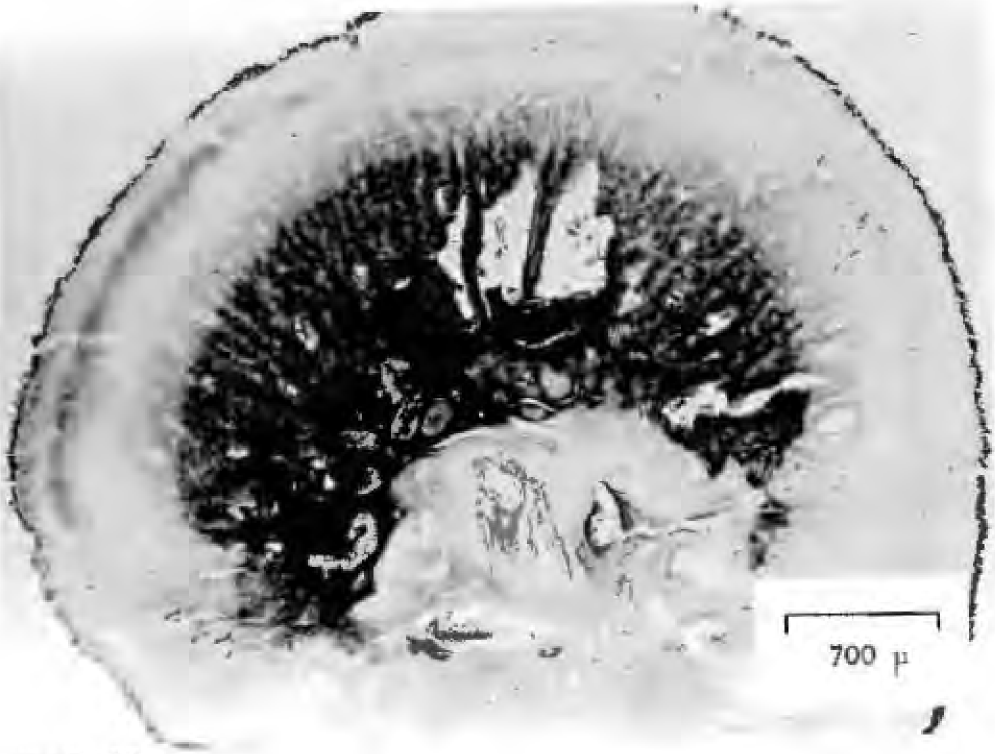

PLATE 18.

PLATE 19.

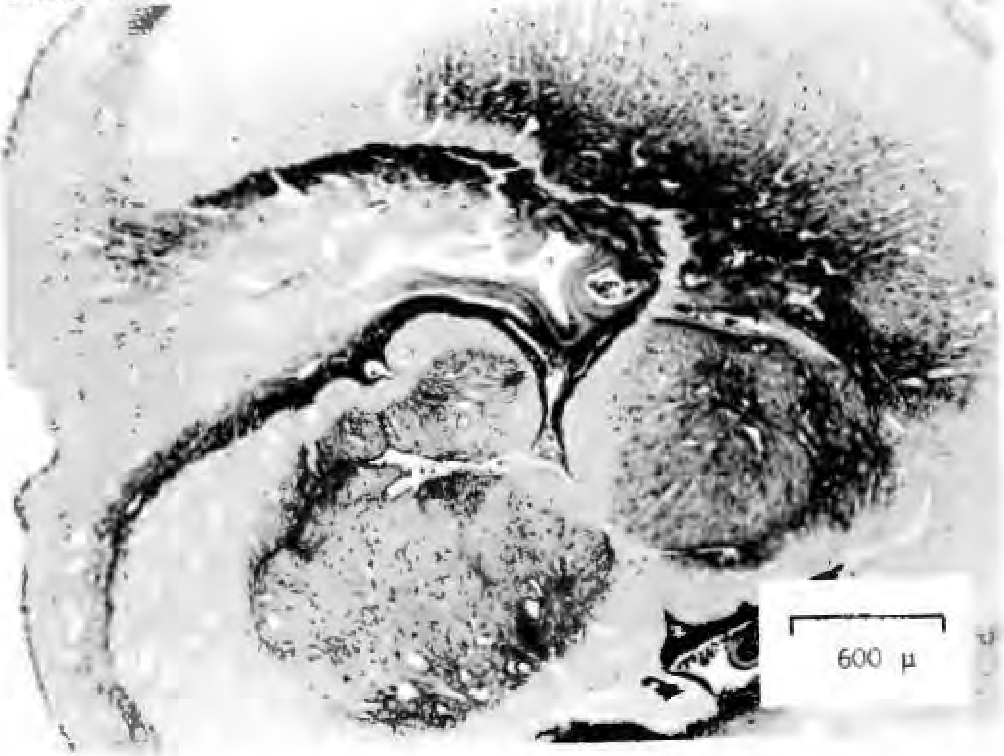

PLATE 18. Silver staining of salt-loaded fish showed high densities of silver deposit in the ducts and tubules leading to the central canal. This is shown here, where the radial arrangement of tubules is apparent.

PLATE 19. Results of silver staining rectal gland tissue after fish had been salt-loaded. Dense silver deposits are indicative of chloride concentrations and in this section individual intracellular deposits are apparent, as well as the dense chloride deposits in ducts leading to the central canal. The arrangement of tubules and ducts into lobules, rather than a definite radial arrangement, is also apparent in this T.S. through the lower region of a gland.

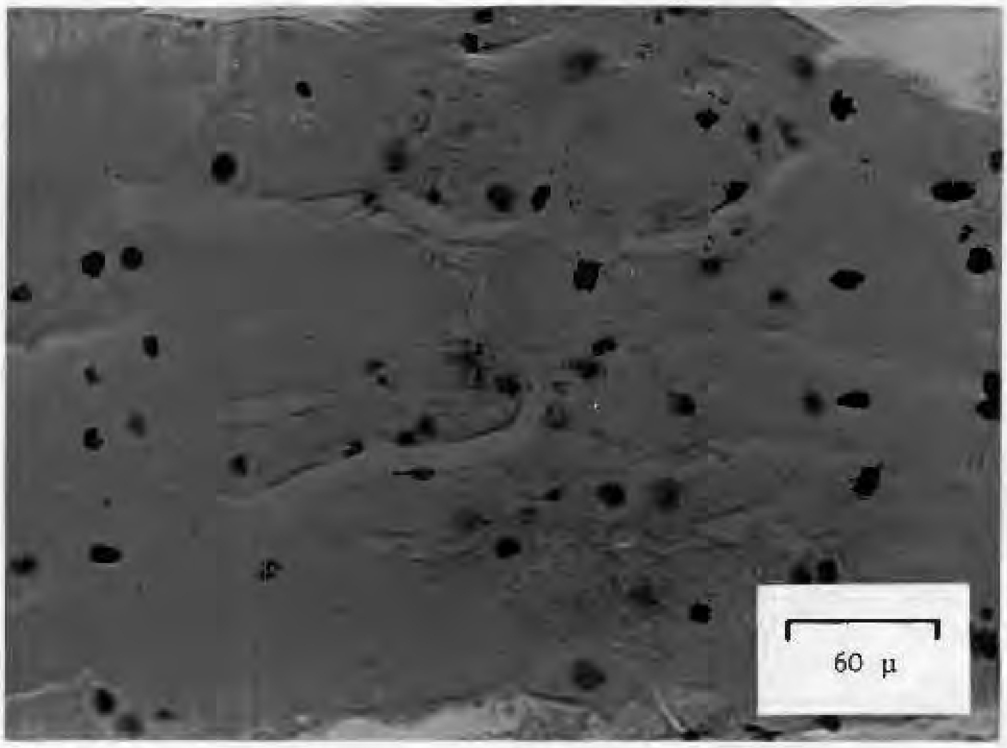

PLATE 20.

PLATE 21.

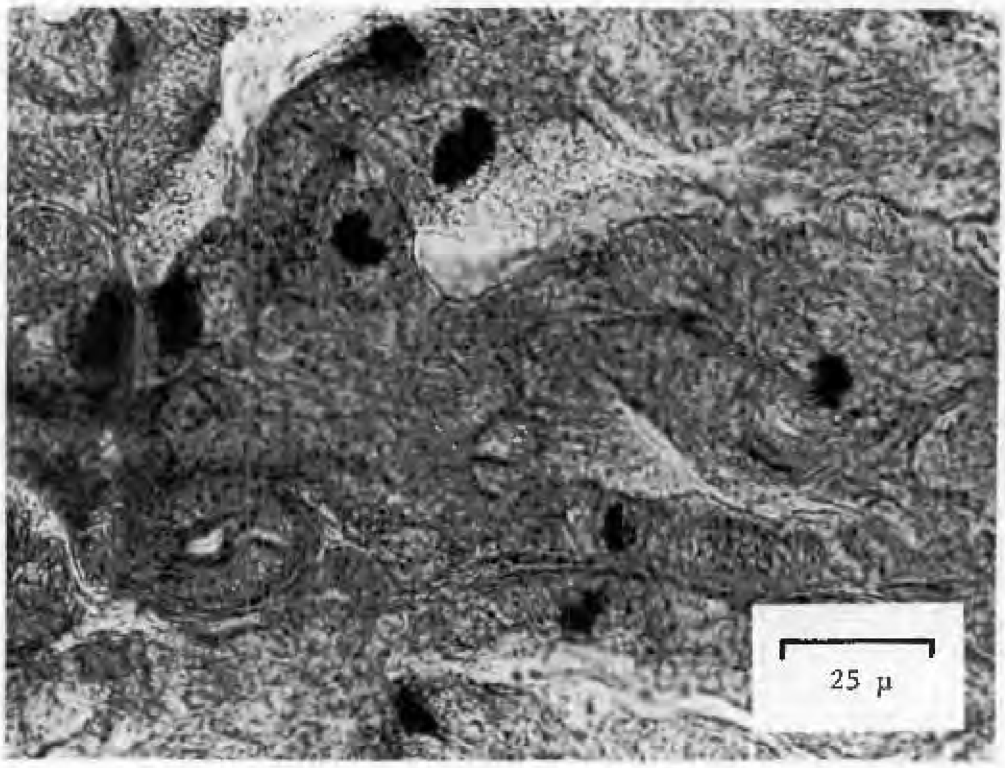

PLATE 20. Silver deposits in the glandular cells, as seen under higher magnification.

PLATE 21. Intracellular silver deposits as seen under high magnification. The appearance of these silver deposits bears striking resemblance to that of silver deposits seen in branchial tissue of this species.

DISCUSSION

The appearance of the tissue after sectioning suggests a radial arrangement of the rectal gland tubules in *Poroderma africanum*, although this is most apparent in the head region of the gland and is not so easily visible in the lower region where the central canal is enlarged. In this region the matrix of the gland appears to be subdivided into lobules, by septa of the glandular parenchyma, although a radial arrangement of tubules is still apparent within each lobule. This preliminary investigation suggests that several collecting ducts are formed in the lower regions of the gland, running parallel with the central canal and presumably joining it before the canal enters the gut. The fine structure of the glands examined, when considered over their entire length, may therefore be considered as non-uniform.

Previous examination of rectal glands (Bonting, 1966) from 9 elasmobranch species has shown that the fine structure tends to be of only 2 different types. A radial arrangement of tubules, presumably over the entire length of the gland, was shown for rectal glands of the spiny dogfish (*Squalus acanthias*). sand shark (*Carcharias littoralis*), smooth dogfish (*Mustelus canis*) and sickle-shaped shark (*Carcharhinus falciformis*), In the rectal glands of the butterfly ray (*Pteroplatea altavela*), eagle ray (*Myliobatus freminvillei*). sting ray (*Dasyatis centrura*) and monkfish (*Squatina squatina*) the tissue was divided up into lobules by thin fibrous septa, so that the tubules were orientated in a longitudinal direction. The septa were found to contain smooth muscle cells and nerve fibres. Such a structure seems very similar to that observed in the lower regions of glands in Poroderma africanum.

With respect to the results of the chloride test on rectal gland tissue, the present investigation can only be termed superficial and under more favourable conditions the examination would habe been taken further, with incorporation of electron-microscopy. In order to qualify the ion transport mechanism operating in the rectal gland tubule cells a more intense investigation and examination of cellular components such as the mitochondria, endoplasmic reticulum and golgi apparatus would be necessary, together with possible enzymatic assays. It is therefore not feasible to suggest a definite 'chloride cell' mechanism present in the rectal gland at this stage, although present results show a marked similarity in appearance between the intracellular silver deposits of tubule cells and those shown previously (Haywood, 1974b) in gill tissue of Poroderma africanum, where the suggestion of 'chloride cells' is supported by other findings.

Previous findings (Haywood, 1974d) have indicated that the rectal gland in Poroderma africanum may regulate body chloride to a greater extent than sodium. After salt loading, normal fish showed slightly greater rises in serum sodium than chloride, but when the rectal gland was ligated, the converse was shown to occur. In all cases ligation of the rectal gland produced only short-term effects on blood salt levels and the role of the gland as an ionic regulator was considered minor in this species.

Doyle (1962) has shown that the salt-transporting system in the rectal gland of the sting ray (Urolophus) is characterised by a series of vesicular components of some complexity and there was reasonably good evidence for the discharge of large numbers of small vacuoles into the lumen of ducts, by fusion of these with the apical cell membranes. examination of tubule cells also showed large numbers of mitochondria, glycogen granules and RNA particles. Bonting (1966) demonstrated the presence of Na-K ATPase in high activity in the rectal glands of 9 species of elasmobranch and further showed that species with a radial arrangement of tubules in the gland had higher enzyme activity per kg body weight than species with the lobular divisions of the glandular parenchyma. He found that many tubular cells contained strongly PAS-positive cytoplasmic granules, some of which were faintly stained with Alcian blue, suggesting that they might be concerned with mucin production, and hence might play a role in the Na-secreting process by binding Na-ions that have been secreted into the tubular lumen and central canal. Bonting suggested that "an additional argument for a role of the Na-K ATPase in salt secretion by the elasmobranch rectal gland can be derived from the histological similarity between this gland (Doyle, 1962) and the marine bird nasal gland (Doyle, 1960)." Further similarity can be derived from the occurrence of regressive changes in both types of gland, upon prolonged disuse (Bonting _et al_., 1964; Oguri, 1964).

Thesleff & Schmidt-Nielsen (1962) were able to abolish secretions of marine bird nasal glands by retrograde injections of ouabain, through the duct of the gland. Since ouabain is a strong specific inhibitor of Na-K ATPase, this was an important argument for a primary role of this enzyme in bird nasal gland secretions. Dr. R. Palmer, Dept. of Pharmacology, University of Florida (Bonting, 1966) was able to show similar inhibition of rectal gland secretion after ouabain administration, and it thus appears that the Na-K

ATPase system of the elasmobranch rectal gland is primarily concerned with salt secretion, indicating an active transport mechanism.

Similar enzyme activity has been demonstrated in isolated 'chloride cells' of fish gills (Kamiya, 1972) and it has now been shown that such cells are also present in elasmobranch branchial tissue (Doyle & Gorecki, 1961; Payan & Maetz, 1973) - indeed they seem present in gill tissue of Poroderma africanum (Haywood, 1974a). In view of this, and the similarity of silver granules apparent in tubule cells (present investigation) to those found in the gill tissue of the same species, it does not seem impossible for similar cells to be present in the tubules of the rectal gland of Poroderma africanum. Such might well account for a marginally greater regulation of body chloride although such cells are likely to transport Na-ions indirectly by ionic attraction.

CHAPTER 10

DISCUSSION AND CONCLUSIONS

The findings of this investigation may be listed as follows :-

(1) In the laboratory, the species tolerates a wide range of external salinities - from about 18.0 to 47.5‰ .

(2) Blood chloride varies from 200 mM/l, in the most dilute medium to 315 mM/l, in the highest salinity, with a mean value of about 260 mM/l in normal sea water.

(3) Both well-fed and underfed animals show similar results, as do both sexes.

(4) Serum osmolarity parallels the isosmotic line over the major part of the tolerated salinity range.

(5) Serum urea concentrations alter with external salinity.

(6) There is a pronounced dietary effect upon both serum urea concentration and osmolarity, over the exposed salinity range. Underfed animals have lower urea concentrations and osmolarities than well fed animals, at the same external salinity.

(7) Animals fed only at monthly intervals exhibit hypo-osmotic serum, yet seem to maintain osmotic equilibrium. Animals fed at intermediate intervals exhibit varying degrees of hypo-osmolarity and since serum urea is found to vary in a similar manner, the final osmolarity can be attributed largely to the

serum urea value.

(8) Serum sodium varies from about 190 to 365 mM/l, over the salinity range tolerated, with a mean serum sodium value of 238 mM/l, in normal sea water.

(9) Again there is no sex or diet effect upon serum sodium values.

(10) In dilute sea water, serum sodium parallels the rise in serum chloride with increase in external salinity, but above about 107% sea water serum sodium begins to rise sharply.

(11) As a result of the above, blood from fish in hyper-saline media show higher serum sodium values than chloride whereas the opposite is true for the blood of fish from dilute sea water.

(12) The exposure of fish to some non-mixohaline solutions, artificially produced in the laboratory, suggests that the various blood ions, especially sodium and chloride, may be regulated independantly of each other, but proportionally to their respective concentrations in the external medium.

(13) Using radio-isotopes, the turnover rates for sodium chloride, water and urea are found to be 0.24, 2,47, 97 and 0.08%/h respectively, for fish in normal sea water.

(14) Chloride and water turnover rates show maximal values in normal sea water and decline with variations of the salinity away from this value, the decline in chloride turnover rate being more marked.

(15) Salinity change is also found to affect urea turnover rates, but in this case turnover is reduced in lower salinities and increased in hyper-saline media.

(16) The exchangeable ionic space has been calculated, as chloride space, as 34.4% body volume or 32.4 ml/100g fresh weight.

(17) Haematocrits show that a sudden drop in the external osmolarity produces an increase in blood volume, measured as a decrease in blood p.c.v.'s (packed cell volume). Similarly, exposure of fish to increased external osmolarity produces rises in blood p.c.v.

(18) Hypo-osmotic fish (due to underfeeding) show greater p.c.v. changes, upon transfer to different medium, than normally-fed fish.

(19) The p.c.v.'s of normally-fed fish return to initial values within 7 days of transfer to the new medium, but hypo-osmotic animals take longer to re-attain osmotic balance.

(20) The use of phenol red demonstrates that normally-fed animals do not drink the medium to any appreciable extent, though hypo-osmotic animals were found to do so.

(21) Animals with surgically-closed rectal gland and urinary systems can be kept successfully, with little change in blood sodium and chloride levels, over a period of 2 weeks in the laboratory, suggesting regulation by some other organ such as the gills.

(22) Both the radio-isotopes Sodium-22 and Chloride-36 are found to be lost at the gills, against the concentration gradient, for fish in normal sea water.

(23) Histochemical studies of gill tissue, incorporating the chloride test, suggest that chloride transport may occur at the gills in this species.

(24) Fish with inoperative rectal glands show only slight rise in blood sodium and chloride levels, as measured over a period of 2 weeks, and compared with control animals.

(25) Salt loading of both control fish and those with inoperative rectal glands demonstrates that blood salt levels return to normal within 7 h of sodium chloride injection.

(26) Results indicate that the rectal gland may control chloride levels more than sodium, although its effect was not found to be very pronounced in either case.

(27) The secretion rate of urine and rectal gland fluid is considered to be very small in this species, since the two collected as a combined fluid only averaged a production rate of 3 ml/kg/day.

(28) Estimation of the cloacal salt loss via urine and rectal gland fluid suggests that cloacal salt loss accounts for only 27.2% total sodium turnover, 14.2% total urea turnover and only 2.6% total chloride turnover.

(29) Results therefore indicate that over 97% of the chloride

loss from the animals occurs at some other site such as the gills, and/or the epithelium.

(30) Histochemical study of rectal gland tissue shows similar intracellular silver deposits to those found in branchial tissue. These deposits are more abundant in the glands of previously salt-loaded animals.

These conclusions indicate that in general the osmoregulation of this species is similar to that of other marine elasmobranchs, although several interesting observations come to light.

Whilst there is no evidence to date that this species invades estuaries or brackish waters, and must therefore be regarded as stenohaline, present results have shown that, under laboratory conditions, it is able to withstand a considerable change in external salinity without apparent adverse effects. It should be noted that the species tends to be more a 'conformer' than a 'regulator' with regard to blood osmolarity, which tends to parallel the external osmolarity, and perhaps it would be true to say that this characteristic is shown more by stenohaline animals than by euryhaline ones. Added to this, these dogfish seem to show reduction in the body permeability to ions and water, when in abnormal salinity - again a possible modification towards stenohaline conditions. Nevertheless, the fact that they are able to tolerate this wide range of salinities is in itself evidence that salinity is probably not the major factor influencing the distribution of this species, under natural conditions.

Results have also demonstrated that turnover of the major ions and water is, in general, similar to that of other marine species, with a ten-fold difference between the turnover rates of chloride and sodium. The exchangeable ionic space, measured as chloride space, seems to be in the same order of magnitude as that for other animals examined, although perhaps a little larger than previous findings. The rectal gland, however, does not play as large a role in the osmoregulation of this species as was previously suggested for other dogfish. On the other hand, whereas the gills seem to have been underestimated as regulatory organs in the past, they appear to play an important part in the general ionic and osmotic regulation of this species and results suggest that a large percentage of salt and water transfer between body fluids and the external medium occurs at the branchial site. An interesting connection between branchial salt regulation and that by the rectal gland may exist, in respect of chloride transport, since histochemical examination of both rectal gland and branchial tissue has indicated the presence of intracellular chloride concentrations which may be indicative of a chloride transport mechanism. One point which seems to have come through clearly is that, for the particular ions assayed, regulation of these by the animal seems to be a separate process for each ion species and appears to be independant of the concentration of other ions present but dependant upon the concentration difference of that ion between internal and external media.

Marine teleosts and elasmobranchs have hitherto been regarded as rather distinct groups, with regard to the physiology of salt and water balance, but present results have shown that, when underfed, dogfish become hypo-osmotic and drink the sea water, as do marine teleosts, probably excreting salts at the gills. Whilst this has only been shown in laboratory animals, and it cannot be postulated how often the situation occurs in the field, the phenomenon itself suggests a closer physiological link between the marine elasmobranchs and teleosts, than was previously thought, and indicates that more physiological investigation into the osmoregulation of other elasmobranch species is called for before any real comparison between the two groups can be made.

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APPENDICES 1 TO 5

APPENDIX 1

ACTUAL VALUES REPRESENTED IN FIGURES 1 TO 4 OF CHAPTER 4.

Figure 1 Serum chloride versus external chloride (mM/1)

Ext. Cl	Serum	Ext. Cl	Serum
290	200	547	252, 260, 266
295	200	550	261, 267
305	195, 202	553	268
317	200	560	265, 272
322	200	565	270, 275
335	200	570	263, 273
360	201, 205, 209	580	273
375	202, 213	590	277
390	206, 212	600	273, 277
405	212, 220	610	274, 287
412	216	625	286
420	215	635	286
425	223	540	287
435	225	645	283, 287, 294, 307
440	223	655	292, 299
450	223, 237	675	305
460	229, 234	690	303
465	231, 250	700	300, 312
482	242	715	307, 317
490	221, 240	730	313
495	242, 247	740	310
507	242	750	314
512	250	765	302, 315, 322
525	252, 257	775	315
545	258		

Figure 2 Serum osmolarities versus external osmolarity. (mOsm/l)

(□ high intake, △ low intake fish)

External	△	□
640	700	
645	680	
650	710	
670	700	720
685		730
695	650, 700 750	
700		750, 730
720	730	775
740	740	780
760	760	805
780	760, 780	820
800	800	860, 880
820	810	845
830		880
860	810, 820 835	890, 905
880	850	920
890	830, 850	930
910	890	
920	870, 900	950
935	900	980
950	930	980
980	880, 890 950	1020
1000	940, 950	1050
1020	980	1060
1030	990	1060
1040	990, 970	1060, 1090
1050	950, 1000	1070, 1110
1065	980, 1010	1110
1085		1130, 1170
1100	1030	1150, 1170
1120	1040, 1070	1170
1140	1060, 1080	1190
1160	1080	1200
1175		1240
1180	1100	1230
1200	1130, 1140	1260
1205		1230
1210	1140	1250
1230	1180	1270
1255	1180	1290, 1310
1275	1200	1330
1280		1320
1300	1210	1340
1320	1240, 1260, 1280	1340, 1370

Figure 3 Serum urea concentrations versus external osmolarity

(O high intake, ● low intake fish)

External osmolarity (mOsm/l)	●	(mM/l) O
700	225, 238	216, 232
760	265, 272	279, 285
775		272
820	280, 290	308, 323
850	275	340
865	340	364
870	310, 314	365
910	313	387
920	317	
935	352	400, 427
980	387	433
990	375	
1000	392	432, 440, 453
1040	405, 410	440, 456 480
1050	357, 398, 402, 407, 416	456, 466, 475
1080	427	
1100	415	452, 487
1150	450	500
1200	482, 490	515, 537, 542
1320	505	550

Figure 4 Variation in serum urea and osmolarity with time.

Days	Serum urea (mM/1)				Serum osmolarity (mOsm/Ml)			
1					1088	1093	1098	1110
4	515	507	496		1086	1077	1079	1100
7	490	480	470	465	1080	1069	1075	1069
10	467	463	-	-	1070	1060	1068	1066
16	424	439	443	434	1040	1048	1035	1030
21	405	424	429	410	1038	1040	1030	1020
26	385	415	425	392	1027	1037	1020	1002
31	397	420	425	400	1027	1037	1022	1010
33	432	420	455	400	1026	1074v	1020	1081

APPENDIX 2

ACTUAL VALUES REPRESENTED IN FIGURES 6, 7 AND 8 OF CHAPTER 5.

FIGURE 6. CHLORIDE TURNOVER RATE (%/H) VERSUS EXTERNAL SALINITY. (mMCl/1)

External Cl	Turnovers	Mean	External Cl	Turnovers	Mean
420	0.95				
	1.05	1.09	600	1.20	
	1.28			1.35	1.38
				1.41	
				1.58	
430	0.87	0.94	640	1.60	1.63
	1.00			1.66	
455	1.10	1.15	650	2.15	
	1.20			1.55	1.80
				1.70	
480	1.10				
	1.28	1.30			
	1.35				
	1.48				
500	1.55				
	1.72				
	1.95	1.89			
	2.05				
	2.20				
520	2.25				
	2.36	2.40			
	2.45				
	2.56				
560	2.40				
	2.30				
	2.15	2.13			
	1.98				
	1.84				
580	0.98				
	1.05				
	1.30	1.31			
	1.42				
	1.50				
	1.65				

FIGURE 7. UREA TURNOVER (%/H) VERSUS EXTERNAL OSMOLARITY (mOsm/l)

External osmolarity	Turnover rate	Mean value
750	0.045	0.056
	0.049	
	0.061	
	0.068	
900	0.066	0.079
	0.074	
	0.079	
	0.084	
	0.093	
1000	0.060	0.074
	0.067	
	0.075	
	0.094	
1030	0.063	0.079
	0.066	
	0.073	
	0.078	
	0.085	
	0.089	
	0.096	
1170	0.085	0.110
	0.092	
	0.109	
	0.118	
	0.145	

FIGURE 8. WATER TURNOVER RATE (%/H) VERSUS EXTERNAL MEDIUM (mM Cl/l)

External medium	Turnover rate	Mean
450	78.2	82.7
	81.0	
	83.0	
	84.5	
	86.8	
465	81.0	84.1
	84.0	
	87.5	
500	76.0	81.3
	80.0	
	82.5	
	86.2	
540	87.0	88.5
	90.0	
550	92.0	97.1
	94.0	
	94.9	
	95.1	
	97.2	
	102.7	
	104.1	
560	90.0	93.2
	92.2	
	93.5	
	97.5	
600	80.0	86.2
	85.0	
	87.0	
	88.2	
	91.0	
650	88.0	85.2
	85.5	
	93.1	

APPENDIX 3

ACTUAL VALUES REPRESENTED IN FIGURE 10 OF CHAPTER 7.

FIGURE 10. SERUM SODIUM VERSUS EXTERNAL SODIUM CONCENTRATION (mM/1)

Ext. Na	Serum Na.	Mean
280	179	
	191	190
	200	
305	192	
	198	
	204	201
	210	
335	199	
	210	208
	215	
370	217	
	225	223
	229	
410	227	231
	235	
435	224	
	230	
	241	234
	243	
467	225	
	230	
	235	
	235	
	239	
	243	238
	243	
	243	
	252	
475	238	
	248	
	257	251
	262	

Ext. Na	Serum Na	Mean
500	238	
	246	248
	252	
	257	
520	251	260
	269	
531	268	272
	276	
550	270	
	280	
	283	285
	289	
	304	
565	294	
	297	302
	304	
	313	
591	311	316
	322	
612	327	
	338	338
	342	
	345	
636	340	
	348	347
	354	
660	340	
	359	358
	362	
	372	

APPENDIX 4

TABLE OF WEIGHTS AND MEASUREMENTS OF RECTAL GLANDS EXAMINED IN CHAPTER 9.

FISH NO.	SEX	FRESH WEIGHT (kg)	RECTAL GLAND		
			LENGTH (cm)	MAX. WIDTH (cm)	FRESH WEIGHT (g)
1		0.872	1.2	0.5	0.33
2		0.920	1.3	0.5	0.35
3		1.17 *	2.0	0.5	0.48
4		1.50 *	2.2	0.6	0.52
5		1.70 *	1.6	0.6	0.26
6		1.80 *	1.6	0.6	0.28
7		2.05 *	2.5	0.7	0.56
8		4.15 *	2.4	0.8	0.83

* Accurate only to 2 decimal places.

APPENDIX 5

COMPOSITION OF THE BLOOD OF _CALLORHYNCHUS CAPENSIS_ DUMERIL (HOLOCEPHALI).*

(Additional publication in support of thesis)

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* Published in "South African Journal of Science" March 1974.

COMPOSITION OF THE BLOOD OF CALLORHYNCHUS CAPENSIS DUMERIL (HOLOCEPHALI)

Concentrations of urea and other substances in the body fluids of holocephalan fishes have long been assumed to throw light on the phylogenetic position and status of the group [1,2]. Virtually all measurements which have been made, however, relate to the family Chimaeridae[2-5], the only work on the blood of the monogeneric family Callorhynchidae being that of Dakin[6] on *Callorhynchus millii*, over 40 years ago.

Dakin found that the concentrations of urea and chloride in the blood of his animals lay well within the range displayed by elasmobranchs and concluded that "there can be no doubt whatever from these figures that the *Holocephali are typical Elasmobranchs*." This conclusion has not been supported by subsequent work on the Chimaeridae, the values obtained for urea concentrations being lower, and those for chloride higher, than those obtained from elasmobranch fishes. In view of these disparate results it was clearly essential that further analyses be undertaken within the family Callorhynchidae.

Callorhynchus capensis is a common holocephalan around the coasts of the Cape Peninsula, South Africa. Moreover, it occurs in relatively shallow water, occasional individuals being caught in the nets of seine fishermen. They also enter the influent pipe of a local power station, becoming trapped in the cooling system, from which they can be removed without damage. Animals were obtained from both these sources, heart-puncture being performed either in the field or in the laboratory and the blood allowed to clot overnight at 8°C. The samples were then centrifuged and the clear serum used to determine osmolarity in a Knauer Osmometer with freezing attachment. Urea concentrations were determined colorimetrically, using the method of Pré *et al.*[7], while chloride was measured by titration against mecuric nitrate, after the method of Schales and Schales[8].

The serum osmolarity of 9 animals removed from the power station on 5/6/73 varied between 1,030 and 1,045 mOsm/1, averaging 1,039. Sea water sampled from the same source at the same time was found to have an osmolarity of 1,040 mOsm/1. Tests on individuals obtained on other occasions, both from the same source and from seine fishermen, confirm that the blood of this species is isomotic with the sea water in which the animals have been living. This conclusion has previously been reached for members of the family Chimaeridae[2,4,5], as well as for *Callorhynchus millii*[6]. Nicol[3] seems to have been mistaken in reporting that the blood of *Hydrolagus* was hyposmotic to sea water; his mistake could have arisen from testing under-fed animals, as Haywood[9] has shown that the blood of the elasmobranch *Poroderma* becomes hyposmotic under such circumstances.

Serum urea values obtained from 12 individuals of *Callorhynchus capensis*, all collected from the power station on the same day, varied between 266 and 315 mM/1, the average figure being 289. Chloride determinations on the same samples gave values ranging between 345 and 370 mM/1, the average being 359. The urea average lies between the values obtained by Urist[4] and Read[2] for *Hydrolagus colliei* and is only slightly higher than that reported by Fange and Fugelli[5] (265 mM/1) for *Chimaera monstrosa*. The average chloride value for *Callorhynchus capensis* is higher than the values reported for *Hydrolagus*[2,4] but is lower than that of *Chimaera monstrosa*[5] (380 mM/1).

We must conclude, therefore, that with respect to urea and chloride concentrations, the blood of *Callorhynchus capensis* resembles that of chimaerids and that it differs from that of typical elasmobranchs, urea values being lower and chloride values higher than are usual in that group. Our results also differ markedly from thos obtained by Dakin[6] on *Callorhynchus millii*; in view of the age of this work and the relatively crude methods used, we believe it should be repeated before any comparison between the two species of *Callorhynchus* is attempted.

G.P. Haywood
A.C. Brown
P.A. Cook

Department of Zoology,
University of Cape Town.

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