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THE EFFECTS OF HYPOPHYSECTOMY ON OSMOREGULATION IN THE EURYHALINE FLOUNDER *PLATICHTHYS FLESUS* (L.)

A thesis submitted for the degree of Doctor of Philosophy of the University of Stirling.

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I declare that the work presented here is, unless due acknowledgement has been made, purely the result of my own investigations and has not been previously presented for examination either in part or as a whole.

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CHAPTER I

INTRODUCTION

INTRODUCTION

Euryhaline teleostean fish inhabit a range of aquatic environments in which the external osmolality varies from less than 1mOsm/kg in freshwater to circa 1000mOsm/kg in sea water. Teleosts in hypoosmotic media are subject to an osmotic influx of water and a passive efflux of electrolytes, whereas in hyperosmotic conditions these fluxes operate in the reverse direction. Against these adverse effects euryhaline teleosts are able to maintain a regulated internal osmolality within the region of 300mOsm/kg. A homeostatic control of water content and electrolyte composition is achieved by the activities of the three osmo(iono-)regulatory organs - the gill, gut and kidney. A scheme for teleostean osmoregulation was proposed originally by Smith (1930,1932) and Krogh (1939). Comprehensive reviews of more recent advances in this field are available (Black, 1957; Potts and Parry, 1964; Parry, 1966; Maetz, 1968,1970a; Potts, 1968; Conte, 1969; Hickman and Trump, 1969; Holmes and Donaldson, 1969). In sea water teleosts an osmotic efflux of water is compensated by the drinking and subsequent intestinal absorption of the external medium. Monovalent ions absorbed from the gut are excreted at the gill and divalent ions are lost primarily via the kidney, which also conserves water. In freshwater teleosts drinking is reduced and the osmotic influx of water is excreted by the kidney, which conserves ions to

produce a copious, dilute urine. The passive efflux and renal depletion of ions is compensated by their active absorption by the gill from the external medium.

As with higher vertebrates neural and endocrine mechanisms are implicated in the control of teleostean osmoregulation. Research into endocrine mechanisms has undergone a marked expansion over the last two decades (reviews: Fontaine, 1956; Hoar, 1957; Pickford and Atz, 1957; Pickford, 1959; Jones and Phillips, 1960; Parry, 1966; Bern, 1967; Maetz, 1968; Ball, 1969a,b; Ball and Baker, 1969; Chester Jones et al, 1969a; Copp, 1969; Henderson et al, 1970; Olivereau and Ball, 1970; Oguri, 1970). Two main approaches have been adopted in investigations on the role of endocrine organs in teleosts: (1) to study the effects of surgical ablation (or biochemical inhibition) of endocrine organs on osmoregulatory function with subsequent attempts at replacement therapy with exogenous (mammalian) hormones. (2) to correlate histophysiological changes in endocrine tissues with osmoregulatory stimuli, essentially variations in external salinity. Other widely used techniques have involved the extraction and assay of hormones in blood and endocrine tissues and studies of the effects of exogenous hormones upon both intact fish and in vitro preparations of their osmoregulatory organs. Recently the more biochemical aspects of osmoregulation have received much attention, with particular reference to the manner in which endocrine factors influence ionic and water transfer at permeable membranes, and also the activity of enzymes involved in ionic transport.

The major teleostean endocrine glands concerned in osmoregulation are the hypophysis and the adrenocortical tissue; the thyroid, parathyroid, ultimo branchial glands, Stannius corpuscles and the urophysis may be implicated to a lesser extent. The neurohypophysial peptides, a prolactin-like factor and an adrenocortico trophic factor are the important hypophysial agents. The endocrine control of osmoregulation is studied most conveniently in euryhaline teleosts since these fish exhibit the different osmoregulation adaptations characteristic of stenohaline marine and freshwater teleosts and also prove excellent experimental animals. Extensive investigations have been made on the killifish, Fundulus heteroclitus, and various eels, Anguilla spp. The European flounder, Platichthys flesus (L.) is strongly euryhaline and has formed the subject of early investigations on osmoregulatory function (Dakin, 1908; Henschel, 1936) and a more recent series of studies using modern techniques (Motais et al, 1966; Lahlou, 1966, 1967; Motais, 1967; Evans, 1969; Maetz, 1969b). However information concerning the role of hormones in this species is rather limited (Motais and Maetz, 1964; Motais, 1967, Motais and Maetz, 1967). In the present study on the flounder the hypophysial control of osmoregulation is investigated using the technique of hypophysectomy, which has not been performed previously in this species.

The investigation is divided into two complementary sections. In Stirling the effects of hypophysectomy on osmoregulation in both sea water and freshwater were

determined by analysis of the electrolyte composition of blood and urine. In Villefranche-sur-Mer (with the Groupe de Biologie marine, Commissariat de l'Energie Atomique) isotopic kinetic techniques were used to investigate the hypophysial control of ionic and water exchanges between the flounder and its environment.

In this thesis the review includes a brief description of teleostean osmoregulatory function with special reference to Platichthys flesus; there follows a comprehensive review of the role of the pituitary gland and its associated hormonal factors in controlling osmoregulation.

C H A P T E R I I

R E V I E W

OSMOREGULATORY MECHANISMS IN TELEOSTS

Intestine

The role of the intestine in the maintenance of water and electrolyte balance has been reviewed recently by Maetz (1970a). In SW-adapted teleosts drinking and intestinal water absorption compensate the osmotic water efflux to the hyperosmotic medium. Contrary to an earlier assertion (Smith, 1930) drinking also occurs in freshwater teleosts (Allee and Frank, 1948) but at a lower rate than in sea water, as demonstrated by studies on euryhaline teleosts (Evans, 1967,1968; Potts and Evans, 1967; Potts et al, 1967; Maetz and Skadhauge, 1968; Motais et al, 1969). External salinity is the dominant factor influencing drinking rate, the latter decreasing as the salinity is reduced (Evans, 1967; Potts and Evans, 1967; Potts et al, 1967; Maetz and Skadhauge, 1968; Dall and Millward, 1969; Lahlou et al, 1969b; Motais et al, 1969). In the flounder the drinking rate is reduced from 192 µl/hr/100 gm in sea water to 37 µl/hr/100 gm in freshwater (Motais et al, 1969).

Ingested sea water may be diluted initially by gut secretion (Skadhauge and Maetz, 1967; Skadhauge, 1969; Maetz, 1970a) then monovalent ions and water are absorbed. The probable mechanism for water absorption is "solute-linked water flow" whereby a local accumulation of ions

within the intestinal epithelium precedes an osmotic water influx to permit water uptake against the prevailing osmotic gradient (Sharrat *et al*, 1964a; Smith, 1964; House and Green, 1965; Aull, 1966; Maetz, 1970a). In *Anguilla* spp. both ionic (Na^+ and Cl^-) and water absorption are increased with adaptation to higher salinities but the increase for water is of greater magnitude indicating an enhanced efficiency in water uptake (Utida *et al*, 1967; Oide, 1967; Oide and Utida, 1967; Skadhange and Maetz, 1967; Skadhange, 1969; Maetz, 1970a). The increase in ionic transport upon SW-adaptation may be correlated with a similarly-induced increase in intestinal activity of Na + K-ATPase, an enzyme concerned in sodium transport (Oide, 1967; Katz and Epstein, 1968; Jampol and Epstein, 1970).

The ions Na^+, K^+ and Cl^- are absorbed preferentially from the teleost intestine together with 60-80% of the ingested water; sodium chloride constitutes 95% of the absorbed salts and a residual fluid rich in Mg^{2+}, SO_4^{2-} and HCO_3^-/CO_3^{2-} remains within the gut lumen (Oide and Utida, 1967; Shehadeh and Gordan, 1969; Hickman, 1969c).

In a SW-adapted teleost absorbing a hyperosmotic fluid from the intestine the excess sodium and chloride ions must be excreted at the branchial epithelium to achieve a net gain of water. In the flounder the excess sodium ions represent an excretion flux of *circa* 100 µeq/hr/100 gm, which is equivalent to the net intestinal influx calculated from the drinking rate (Maetz, 1969b). In FW-adapted teleosts the intestine is unimportant as a source of sodium ions since the sodium content of fresh water is low

(<1 meq/1); the food is a dispensable source of electrolytes since starved fish survive well in freshwater.

Kidney

The kidney function of teleosts has been reviewed recently by Hickman and Trump (1969). In SW-adapted teleosts the major renal functions are water conservation and the excretion of divalent ions absorbed from the intestine. The glomerular filtration rate is low and the active reabsorption of water in the renal tubule further reduces urine flow. The reduction in urine flow accompanying the FW→SW transfer of teleosts may exceed 90% (Holmes, 1961; Holmes and McBean, 1963; Stanley and Fleming, 1964; Fleming and Stanley, 1965). Variations in GFR, and hence in urine flow, are achieved primarily by changes in the number of active glomeruli rather than in their individual filtration rate (Mackay, 1967 cited Hickman, 1968a; Lahlou, 1966, 1967; Hickman, 1968a) but the superimposed effects of tubular water reabsorption frequently obscure a direct proportionality between GFR and urine flow. Mg^{2+} and SO_4^{2-} are the major divalent ions excreted in the urine of SW-adapted teleosts, since 20% of the ions ingested may be absorbed from the intestine. Divalent ions are actively secreted from the tubule; the contribution from plasma filtration is low. The monovalent ions Na^+, K^+, Cl^- are actively reabsorbed (Hickman, 1968b,c). In the flounder the renal sodium flux of 2-4 µeq/hr/100 gm is negligible before total sodium efflux of 2,600 µeq/hr/100 gm (Lahlou, 1967; Motais, 1967).

In FW-adapted teleosts the major renal function is water excretion; the GFR and urine flow are high. Tubular secretion of magnesium and sulphate ions ceases and monovalent ions are conserved by an enhanced tubular reabsorption (Hickman and Trump, 1969). In the flounder GFR is increased from 0.240 ml/hr/100 gm in sea water to 0.416 ml/hr/100 gm in freshwater; tubular reabsorption of water is reduced from 75% to 60%. The reabsorption of monovalent ions is increased only slightly in freshwater to remain within the range, 80 - 90% (Lahlou, 1967,1970). The renal sodium flux of circa 6 μeq/hr/100 gm represents approximately one-third of the total sodium efflux (Lahlou, 1967; Motais, 1967).

Lahlou (1967) found that the urinary bladder of the flounder possesses a reabsorptive function. In sea water this organ reabsorbs both water and sodium, thus effecting a net gain in water since the excess sodium ions are presumably excreted at the gill; in freshwater sodium reabsorption within the bladder reduces renal ion depletion, thereby favouring electrolyte balance.

Gill

The gill is the major osmo(iono-)regulatory effector organ in teleosts. In hyperosmotic and hypoosmotic media electrolyte balance is maintained by the net transfer of ions via the branchial epithelium against the prevailing osmotic gradient. Early work postulated a salt-secretory function for the gill-buccal region of marine teleosts (Keys, 1931; Keys and Willmer, 1932; review, Parry, 1966).

More recently, isotopic kinetic studies have permitted the quantitative evalution of unidirectional ion fluxes across the branchial epithelium (Mullins, 1950; Garcia Romeu and Maetz, 1964; Maetz and Garcia Romeu, 1964; Motais et al, 1965, 1966; Motais and Maetz, 1965; Motais, 1967; Potts and Evans, 1967; Potts et al, 1967; Maetz, 1968, 1969b, 1970a,b; Maetz et al, 1969a). Maetz (1970a) has recently reviewed branchial mechanisms of osmoregulation.

In SW-adapted teleosts internal (exchangeable) sodium is exchanged with external sodium at a rate of 25-50%/hr (Motais et al, 1966; Maetz, 1970a). The major part of this exchange operates across the branchial epithelium and its component fluxes have been studied in the flounder (Motais et al, 1966; Motais, 1967; Maetz, 1969b). The net efflux component (which compensates for intestinal sodium absorption) operates through an Na:K exchange pump, internal sodium being exchanged against external potassium (Maetz, 1969b). The net efflux (circa 100 μeq/hr/100 gm) constitutes only a small proportion of the total efflux (circa 2600 μeq/hr/100 gm) of which the major part (85-90%) operates by Na:Na exchange diffusion (Motais et al, 1966). The exchange diffusion concept (Ussing, 1947) implies a coupling of the two unidirectional fluxes (influx and efflux) through a common carrier, and accounts for the extensive reduction (85-90%) in sodium efflux on abrupt SW→FW transfer, whereupon influx is virtually abolished in the low sodium (< 1 meq/l) medium. The Na:K and Na:Na exchange components are linked through the same carrier,

and the affinity of the latter for potassium greatly exceeds that for sodium (Maetz, 1969b). Apart from the sodium pump (Na:K) and exchange diffusion (Na:Na) components of sodium exchange, there exists a third, purely passive component, which accounts for *circa* 10% of the branchial sodium exchange. Chloride exchange also exhibits an exchange diffusion effect and operates independently of, and at a lower rate than, sodium exchange (Motais, *et al*, 1966); the mechanism of the active chloride extrusion component has not been elucidated.

Recent investigations (Mayer and Nibelle, 1970; Rankin, 1971) have shown that the branchial sodium efflux of the eel may be varied by *in vitro* and *in vivo* manipulations of the internal sodium levels quite independently of sodium influx. These findings indicate that the exchange diffusion concept for branchial sodium exchange in SW-adapted teleosts may be due for revision.

In FW-adapted teleosts total ionic exchange is markedly below the rates observed in sea water; in the flounder the sodium turnover is 0.4%/hr, and the major part of the exchange still operates at the branchial level (Motais *et al*, 1966; Motais, 1967). The branchial sodium influx of freshwater teleosts exceeds efflux by 20-50% to compensate for the renal ion depletion. A high ratio for influx/Na_{ext} indicates that sodium absorption pump has a marked affinity for external sodium; in contrast, efflux/Na_{int} is low implying a reduced passive branchial sodium permeability (Maetz, 1970a). The external ions Na^+ and Cl^- are absorbed independently being exchanged

for internal NH_4^+ and HCO_3^- respectively (Krogh, 1939; Garcia Romeu and Maetz, 1964; Maetz and Garcia Romeu, 1964). An exchange of external Na^+ against internal H^+ may also operate (Kirschner, 1970).

The gill also displays functional variations in relation to water balance. The water permeability of the branchial epithelium is higher in freshwater than in sea water (Potts et al, 1967; Evans, 1969; Motais et al, 1969; Potts and Fleming, 1970). This variation in permeability ensures that the osmotic water flux is less in sea water than in freshwater even though the osmotic gradient in the marine environment exceeds that in freshwater (Motais et al, 1969; Maetz, 1970a).

HYPOPHYSIAL CONTROL OF OSMOREGULATION IN TELEOSTS

HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM

Neurohypophysial Principles of Teleost Fishes

The occurrence and identification of teleost neurohypophysial peptides has been reviewed recently by Sawyer (1966) and Perks (1969). Heller (1941) demonstrated the marked frog water-balance activity of Gadus sp. pituitary extracts and Pickering and Heller (1959) isolated this pressor and antidiuretic factor from an oxytocin-like principle by paper chromatography. Sawyer et al (1959, 1961) suggested that the bioassay activity of Pollachius virens extracts was produced by the presence of arginine vasotocin and oxytocin. Further investigations upon pituitary extracts from a variety of teleosts involving chromatographic extraction, bioassay, analysis of amino-acid sequence and activity comparisons with synthetic analogues have demonstrated that the frog water-balance principle is identical with 8-arginine vasotocin (Katsoyannis and du Vigneaud, 1958, 1959; Heller and Pickering, 1960, 1961; Acher et al, 1961, 1965, 1968; Follet and Heller, 1964; Chauvet et al, 1961; Rasmussen and Craig, 1961; Wilson, 1968; Wilson and Smith, 1968).

The teleostean o ytocin-like principle (Pickering and Heller, 1959, Sawyer et al, 1959, 1961; Acher et al, 1961; Chauvet et al, 1961; Heller and Pickering, 1961) has been

identified by similar methods as 4 serine, 8 isoleucine oxytocin or isotocin (Acher et al 1962, 1965, 1968; Gutmann et al, 1962; Jöhl et al, 1963; Sawyer and van Dyke, 1963; Sawyer and Pickford, 1963; Follet and Heller, 1964; Wilson, 1968; Wilson and Smith, 1968).

Morphology

Perks (1969) has recently made a comprehensive review of the literature relating to the structure of the teleost neurohypophysial system, and to the nature and function of the neurohypophysial peptides.

The preoptic nuclei of the teleost hypothalamus lie dorsal to the optic chiasma and lateral to the third ventricle. The nuclei are divided into two regions - the ventral pars parvocellularis and the dorsal pars magnocellularis which consists of large neurosecretory cells. The elementary neurosecretory vesicles or granules are produced by the Golgi complex of the cells. These vesicles are of two types which may represent the two neurohypophysial peptides of teleosts (Lederis, 1962; Leatherland and Dodd, 1967).

The main route for neurosecretion from the hypothalamus is the preoptico-hypophysial tract. The axons of the tract pass out laterally from the nucleus, then bend ventrally and pass along the infundibular floor. The main tract enters the pituitary gland medially in the caudal region to form the pars nervosa or neurohypophysis. Neural tissue is distinct from adenohypophysial components in juvenile teleosts but in adult fish may penetrate throughout all regions of the adenohypophysis. Frequently the axons form

digitate processes that penetrate the pars intermedia. These neural processes often contain myelinated nerve fibres, ganglion cells and radially arranged pituicytes. Neurosectory fibres pass between the pituicytes to form the main store of neurosecretion in the posterior processes (Dodd and Kerr, 1963; Knowles and Vollrath, 1965 a,b). The stored material consists of granules similar to those of the preoptic nucleus (Lederis, 1962; Knowles and Vollrath, 1966a) and as before the granules are of two sizes. Lederis (1962) found that AVT is associated with the smaller size of granule in Gadus morhua but the source of the other peptide is unconfirmed.

Neurosecretory axons terminate in swellings containing synaptic vesicles. The terminals are applied to pituicytes, pars intermedia cells, the outer intervascular channel of the digitate process or around capillaries. (Knowles and Vollrath, 1966a, Leatherland et al, 1966). The intervascular channel is the main terminal site in the eel and this channel is surrounded by cells of the pars intermedia or may envelope capillaries of the pars nervosa. Capillaries directly associated with the secretory neurones may pass into the systemic circulation or into the adenohypophysis (Henderson, 1969; Hill and Henderson, 1968). Routes therefore exist for the carriage of neurosecretory products direct to the tissues or to influence adenohypophysial function.

Neurosecretory activity in relation to external salinity

Transfer of both SW and FW-adapted teleosts to hyperosmotic saline media resulted in a depletion of neurosecretory material from the preoptic nuclei and neurohypophysis (Arvy

and Gabe, 1954; Arvy et al, 1959; Fridberg and Olsson, 1959; Kawashimo and Hirano, 1968). In Salmo irideus short term transfer from freshwater to sea water was accompanied by a release of material at the axon terminals of the nerohypophysis and an increase in neurosecretory activity in the preoptic nucleus. Bioassay indicated a 50% reduction in pituitary arginine vasotocin but no alteration in 4-Ser, 8-Ileu oxytocin content. After longer periods (4-8 hr) in sea water the peptide content and granulation of the preoptic nuclei of the neurohypophysis was similar to that of freshwater controls (Lederis, 1963, 1964). In Salmo gairdnerii short term transfer depleted arginine vasotocin and raised 4-Ser, 8-Lleu oxytocin content with a return to control levels within six hours (Carlson and Holmes, 1962).

Recently Olivereau (1969b) observed a depletion of neurosecretory material from the preoptico-hypophyseal tract of A. anguilla and A. japonica upon FW→SW transfer in confirmation of earlier findings (Arvy et al, 1954; Schiebler and Hartmann, 1963). Repletion of material occurred more rapidly in the preoptic nuclei than in the neurohypophysis. Using an in situ staining technique Leatherland and Dodd, (1969) found that exchange of eels between FW and SW and short-term salt or water-loading did not have any effect on the staining properties of the neurosecretory tract, but a reduction was observed in various stressful situations (e.g. background colour variation, cold temperature shock). These authors emphasized the difficulties in interpreting changes in staining properties and their correlation (if any) with salinity variation. Alterations in neurosecretory

activity could be detected but not the sign of that change since a decrease in stain-uptake could represent an active system with a rapid turnover of neurosecretory material whereas an increase might indicate storage. A more reliable technique involving autoradiography after uptake of radioactive-labelled amino-acids indicated an increase in neurosecretory activity in freshwater eels and in eels after SW→FW transfer, over levels in sea water eels.

Osmoregulatory effects of neurohypophysial peptides

The effects of exogenous neurohypophysial peptides and pituitary extracts active in these principles upon teleost osmoregulation has been reviewed by Pickford and Atz (1957); Dodds et al (1966), Sawyer (1966), Maetz (1963, 1968) and Perks (1969). Mammalian and teleostean peptides are ineffective in promoting freshwater survival in hypophysectomised F. heteroclitus and Xiphophorus maculeatus or in decreasing extrarenal sodium depletion in operated Poecilia latipinna (Pickford et al, 1965; Schreibman and Kallman, 1966; Ball and Ensor, 1967). Injection of these principles, whether extracted or synthetic, into teleosts is not followed by the typical water balance response (hydro-osmotic effect upon skin and bladder, renal water retention) that is observed in anuran amphibians (Fontaine and Raffy, 1950; Fontaine, 1956; Maetz, 1963; Heller and Bentley, 1965).

In teleosts variations in renal function have been noted in response to treatment with neurohypophysial peptides. Antidiuretic effects were observed in Salmo gairdnerii (Holmes, 1961, cited Heller and Bentley, 1965)

and to a slight extent in Carassius auratus treated with isotocin (Maetz et al, 1964). More typical are the strong diuretic responses found in Carassius auratus (Sexton, 1955; Maetz, 1963; Maetz et al, 1964), Anguilla anguilla (Butler, 1966; Chester-Jones et al, 1969b; Rankin et al, 1967) and Salmo gairdnerii (Holmes and McBean, 1963). The diuresis in the freshwater eel and goldfish is produced by an increase in GFR, demonstrated in the eel by a marked increase in inulin and PAH clearance rates. Urine sodium and chloride levels remain unaltered leading to an increase in renal electrolyte depletion. The rise in GFR but constant urine sodium may imply that the number of active glomeruli is increased without any alteration in the tubular reabsorption of water or electrolytes (Maetz, 1963; Hickman, 1965; Maetz et al, 1964; Lahlou, 1966, 1970; Maetz & Rankin, 1969). Alternatively renal arteriolar vasodilation may increase filtration at glomeruli (since in the eel dorsal aortic blood pressure falls in response to peptides) whilst an increase in sodium reabsorption maintains urine sodium at constant levels (Chester-Jones et al, 1969b). In the goldfish arginine vasotocin (AVT) is more active than isotocin in producing a diuresis, but the converse situation exists in the eel. In the aglomerular teleost Opsanus tau treatment with AVT did not produce a diuresis (Lahlou et al, 1969a).

Lahlou and Giordan (1970) found that AVT (but not isotocin) reduced branchial water exchange in the goldfish as well as having diuretic activity. In the hypophysectomised fish the peptide increased urine flow and reduced the sodium content. Isotocin had a similar effect upon sodium levels in operated fish but altered urine flow so that

renal losses of sodium were reduced (Lahlou, 1970). Neither AVT nor isotocin affect renal function in the flounder Platichthys flesus, whether in sea water or freshwater (Lahlou cited Maetz, 1968 and Maetz & Rankin, 1969).

Neurohypophysial peptides also influence extrarenal sodium fluxes, presumably by an action upon the gill. In the goldfish in freshwater oxytocin, isotocin and AVT increase sodium influx, whilst oxytocin and AVT also promote efflux (Maetz & Julien, 1961; Maetz, 1963; Maetz et al, 1964). In the earlier experiments high doses of oxytocin giving atypical long term responses were used, but later transitory responses were obtained with physiological doses of peptides. AVT augments both sodium influx and efflux at the gill, and also renal sodium depletion resulting in a negative ion balance in the goldfish. Isotocin has a more marked effect upon branchial fluxes and reduces renal loss producing a positive sodium balance. In the flounder adapted to sea water oxytocin (high dose) increases sodium turnover. Oxytocin and AVT (low dose), but not lysine vasopressin, accelerate the increase in branchial sodium efflux that is associated with readaptation to sea water after a sojourn in freshwater (Motais and Maetz, 1964; Motais, 1967; Motais and Maetz, 1967).

Maetz and Rankin (1969) advocated a cautious interpretation of data from experiments with peptides that utilize mammalian rather than teleostean principles (AVT and isotocin), involve pharmacological rather than physiological doses, or use fish where endogenous peptide secretion is not suppressed. Peptide action may be mediated by adenohypophysial hormones especially where long term responses are observed (Sawyer, 1967; Maetz, 1963) although hypophysectomised goldfish respond

to treatment with peptides (Lahlou, 1970; Lahlou and Giordan, 1970). Recent investigations have revealed that neurohypophysial principles exert important haemodynamic effects that influence water and ion fluxes in the gill and kidney.

In Anguilla anguilla the increase in GFR and urine flow in response to isotocin is accompanied by a transient vaso-depressor effect upon the dorsal aorta, but arteriolar vasodilation may produce the increase in filtration (Chester-Jones et al., 1969b). There is a close relationship between the vasoactivity and diuretic potency of peptides in the eel (Rankin, 1967, Chan, 1967, cited Maetz and Rankin, 1969). Sawyer (1966b) associated the vasopressor action of AVT upon the dorsal aorta of the lungfish, Protopterus aethiopicus, with the diuresis and increase in GFR produced by the peptide. AVT is also a potent vaso-pressor agent in Opsanus tau but the absence of a diuresis in this fish is due to the aglomerular kidney (Lahlou et al., 1969a).

Maetz and Rankin (1969) found that neurohypophysial peptides altered the pattern of blood flow through the gill. A high dose of AVT in the FW eel in vivo had a marked pressor effect upon the afferent vessels and slight effect upon the efferent circulation, producing an increase in the dorsoventral pressure difference. An increase in the capillary resistance of the gill was apparent. Such a haemodynamic effect could explain the reduction in branchial water exchange observed in the goldfish treated with AVT (Lahlou and Giordan, 1970). Experiments with isolated perfused eel gills using low doses of peptides confirmed the in vivo response to vasotocin. Isotocin, AVT and oxytocin

(in order of decreasing potency) reduce the perfusion rate of the preparation suggesting possible modifications of the surface contact between internal and external milieux that would affect ionic exchanges. Using the model for gill circulation proposed by Steen & Kruysse (1964) Maetz and Rankin suggested that AVT may divert the main blood flow from the lamellar compartments to the central compartments of the filament. The chloride cells (Keys & Willmer, 1932; review Parry, 1966) presumably active in ion exchange are located upon the sides of the central compartment at the base of the lamellae. Irrigation of these cells is therefore enhanced by AVT and alterations in sodium fluxes in response to peptides may reflect such haemodynamic effects.

HYPOPHYSECTOMY

Effect Upon Survival

Pickford (1953) noted that the hypophysectomised killifish Fundulus heteroclitus was unable to survive in freshwater. Earlier reports had been equivocal, claiming survival (Matthews, 1933, 1939; Abramowitz, 1937) as observed for the hypophysectomised eel Anguilla anguilla in freshwater (Fontaine et al, 1949). Burden (1956) confirmed Pickford's findings by demonstrating that ablated Fundulus heteroclitus survived only 6-7 days in freshwater at 15°C. Failure was accompanied by asthenia, an increase in body weight (6-7%) and a reduction in serum chloride content. Replacement therapy with various mammalian pituitary hormones was ineffective but injections of a brei of killifish pituitaries maintained survival. Perch (Perca flavescens) glands were partially effective but not those from pollack (Pollachius virens). It has since been discovered that prolactin is the sole hormone that promotes freshwater survival (Pickford and Phillips, 1959; Pickford et al, 1965).

Extensive data upon the effects of hypophysectomy upon freshwater survival in numerous teleostean species are summarised in Table 1. A few of the earlier reports lacked precise information concerning the maintenance media and the possible causes of "high post-operative mortality", or employed an inadequate technique to confirm the success

TABLE 1

Freshwater survival of hypophysectomised teleosts (derived and revised from Pickford and Atz, 1957; Schreibman and Kallman, 1966, 1969)

SPECIES	HABITAT	SURVIVAL IN FW	AUTHOR
ORDER CYPRINIFORMES			
Carassius auratus	FW	3 weeks 4 months	Chavin, 1956 Yamazaki, 1961
Phoxinus phoxinus	FW	6 weeks 3 weeks	Giersberg, 1932 Healey, 1940
Rhodeus amarus		Indefinite	Bretschneider & Duyvene de Wit, 1941
Couesius plumbeus	FW	Indefinite	Ahsan, 1966
ORDER SILURIFORMES			
Ameiurus melas	FW	70% survival, 1 month 20% survival, 6 months	Osborn, 1941
Heteropneustes fossilis	FW	Indefinite	Sundararaj, & Goswami, 1965
Ameiurus nebulosus	FW	several months 12 days 1 month	Abramowitz, 1937 Parker, 1941 Viel, 1937
ORDER ANGUILLIFORMES			
Anguilla anguilla	eury-haline SW/FW	2-3 weeks 2 months 18 months 6 weeks	Fontaine et al, 1949 Vilter, 1945 Oliverean & Fontaine, 1965 Butler, 1966
Anguilla rostrata	eury-haline SW/FW	3-4 months	Parker, 1945

ORDER ANTHERIFORMES/..

TABLE 1 (Continued)

SPECIES	HABITAT	SURVIVAL IN FW	AUTHOR
ORDER ANTHERIFORMES			
Fundulus heteroclitus	eury-haline brack-ish	max. 206 days (high post-operative mortality)	Matthews, 1933, 1939
		asthenia 4 days	Pickford, 1953; Pickford et al, 1965
		death 6-7 days	Burden, 1956
Fundulus majalis	halo-philic	limited per-iod	Griffith, 1969
Fundulus rathbuni	FW	extended	Griffith, 1969
Fundulus notatus	FW	extended	Griffith, 1969
Fundulus kansae	eury-haline SW/FW	Indefinite (requires ext. Ca^{2+})	Stanley & Fleming, 1966a Pickford et al, 1966b
Poecilia latipinna	eury-haline SW/FW	1 - 2 days	Ball & Olivereau, 1964; Ball & Ensor, 1969
Poecilia formosa	FW	1 - 2 days	Ball & Kallman, 1962
Poecilia reticulata	FW	5 days	Jalabert & Billard, 1968
Poecilia spp.	mainly FW	2 - 6 days	Schreibman & Kallman, 1969
Xiphophorus spp.	FW	1 - 2 weeks	Schreibman & Kallman, 1966, 1969
Gambusia sp	brack-ish/FW	15-20 days	Chambolle, 1964, 1966
Gambusia affinis	eury-haline SW/FW	6 days (mean)	Schreibman & Kallman, 1969
Aplocheilus sp.	mainly FW	9 days (mean)	Schreibman & Kallman, 1969
Nothobranchius gueritheri/....			

TABLE 1 (Continued)

SPECIES	HABITAT	SURVIVAL IN FW	AUTHOR
ORDER ANTHERIFORMES (Continued)			
Nothobranchius guerítheri	mainly FW	9 days (mean)	Schreibman & Kallman, 1969
Rivulus harti	mainly FW	5 days (mean)	Schreibman & Kallman, 1969
Rivulus micropus	mainly FW	8 days (mean)	Schreibman & Kallman, 1969
Xenotoca eiseni	mainly FW	6 days (mean)	Schreibman & Kallman, 1969
ORDER OPHIOCEPHALIFORMES			
Ophiocephalus punctatus	FW	high post-operative mortality (44 days - 4 fish only)	Belsare, 1965
ORDER PERCIFORMES			
Tilapia mossambica	euryhaline SW/FW	6 days	Handin et al, 1964
Betta splendens		circa 1 week	Schreibman & Kallman, 1965
ORDER SALMONIFORMES			
Salmo gairdnerii	euryhaline SW/FW	Indefinite	Donaldson & McBride, 1967

of hypophysectomy and the absence of hypophysial tissue remnants.

It has been suggested that teleosts may be divided into two categories according to their ability to survive in freshwater following hypophysectomy (Schreibman and Kallman, 1966,1969). These authors pointed out that many of the species tolerant to freshwater are members of the Superorder Ostariophysi (e.g. Orders Cypriniformes, Siluriformes) whereas species that succumb belong mainly to the Order Antheriformes. However it is difficult to relate phylogenetic considerations and/or natural habitat to the pituitary-dependent or independent freshwater survival of teleosts. Amongst the Antheriform genus Fundulus some species (notably Fundulus kansae and Fundulus diaphanus) survive indefinitely in freshwater after hypophysectomy (Pickford and Ball, unpublished, cited Pickford et al, 1965; Stanley and Fleming, 1966a; Griffith, 1969). Teleosts from other taxons whether normally stenohaline or euryhaline may survive (Anguilla anguilla Anguilliformes, Olivereau and Fontaine, 1965; Salmo gairdnerii, Salmoniformes, Donaldson and McBride, 1967) or die in freshwater (Tilapia mossambica, Betta splendens, Perciformes, Handin et al, 1964; Schreibman and Kallman, 1965). Furthermore each hypophysectomised teleost so far investigated has exhibited osmoregulatory defects (particularly electrolyte depletion) in the hypoosmotic environment. These observations, made irrespective of survival ability, suggest that pituitary-dependent and independent teleosts reflect a gradation in the response of a common osmoregulatory mechanism to pituitary ablation, or alternatively, their degree of tolerance to internal dilution and osmotic shock may vary.

Hypophysectomy does not impair the survival of teleosts maintained in sea water, one-third sea water or teleostean Ringer (Pickford, 1953; Burden, 1956; Ball and Olivereau, 1964; Handin et al, 1964; Schriebman and Kallman, 1966, 1969) but this observation may depend to some extent upon the salinity tolerance of the intact teleost (Griffith, 1969).

Effects Upon Osmoregulation

In Sea Water

In SW-adapted fish hypophysectomy led to increases in the levels of plasma sodium and chloride in Anguilla anguilla (Butler, 1966), and in plasma sodium in Poecilia latipinna (Ball and Ensor, 1969). In Fundulus kansae serum and whole body sodium were increased after ablation of the pituitary (Stanley and Fleming, 1967b). In contrast serum chloride content was decreased by hypophysectomy in the related Fundulus heteroclitus in sea water (Pickford et al, 1970a).

Butler (1966) suggested that the increase in sodium content in the eel reflected a decrease in the net branchial efflux of sodium. Stanley and Fleming (1966b, 1967b) showed that extra-renal sodium excretion was less efficient in operated Fundulus kansae in sea water than in intact fish. SW-adapted Fundulus heteroclitus and Anguilla anguilla exchange internal sodium with the environment at a rate equivalent to 27-35% and 33%/hr respectively (Maetz et al, 1967 a,b). After hypophysectomy the rate of exchange is halved in both species. Maetz et al (1967a) suggested that

the major effect was upon the branchial sodium exchange since renal and skin fluxes are negligible by comparison. Despite the increase in plasma sodium observed in Poecilia latipinna (Ball and Ensor, 1969) no change in sodium turnover rate was detected in this species ten days after removal of the pituitary gland (Ball unpublished, cited Olivereau and Ball, 1970).

The content of the enzyme Na + K-ATPase in teleost osmoregulatory organs is correlated with requirements for ion transport. Enzyme activity increases in parallel with sodium exchange in the gills and gut and may decrease in the kidney with a reduction in renal sodium retension in SW-adapted Fundulus heteroclitus, Anguilla rostrata, Anguilla japonica and Oncorhynchus kisutch (Epstein et al, 1967, 1969; Oide, 1967; Kamiya & Utida, 1968, 1969; Jampol & Epstein, 1970; Zaugg and McLain, 1970). Hypophysectomy reduced the content of Na + K-ATPase in the gill of Fundulus heteroclitus (Epstein et al, 1967) and Anguilla anguilla (Milne et al, 1971) but had no effect in Anguilla japonica (Utida et al, 1966). In Anguilla japonica the ablation did not affect the ability of isolated gills to adapt their sodium transport activity to a sea water environment (Hirano et al, 1967), but did reduce the ability of the gut to absorb salt and water (Hirano and Utida, 1968).

Hypophysectomy had no effect upon renal function in eels transfered from fresh water to sea water (Butler, 1966). Urine flow and electrolyte content were similar in operated and intact eels three weeks after transfer. Stanley and Fleming (1966a,b) observed that the rate of adjustment of urine flow and sodium excretion in Fundulus kansae after

transfer to sea water was lower in hypophysectomised fish than in their controls. This effect was temporary since renal function was not affected in fish adapted to sea water (Stanley and Fleming, 1967b). Operated fish did not appear to possess the rare ability to secrete a hyperosmotic urine previously observed in this species (Stanley and Fleming, 1964).

Recently Potts and Fleming (1970) have observed that hypophysectomy led to a slight decrease in tritiated water turnover in Fundulus kansae in hyperosmotic media. This difference was markedly increased in fresh water. At present few data concerning the effects of pituitary ablation upon water permeability in teleosts in sea water are available but Payan and Maetz (1970) have reported an extensive reduction in diffusional water permeability at the gill, and also in urine flow in the elasmobranch Scyliorhinus canalicula after hypophysectomy.

In Freshwater

Many recent reviews have included discussions of the effects of hypophysectomy upon hypo-osmoregulation in teleosts (Maetz, 1968; Ball, 1969a,b; Ball and Ensor, 1969; Conte, 1969; Olivereau and Ball, 1970; Olivereau and Lemoine, 1969).

Since Burden's original observation (1956) that hypophysectomised Fundulus heteroclitus dying in freshwater had a serum chloride concentration 50% less than intact fish reductions in serum osmolality and/or plasma electrolytes have been demonstrated in many ablated teleosts irrespective of their ability to survive in freshwater for long periods.

Reduced osmolalities and plasma sodium or chloride levels have been observed in Fundulus heteroclitus (Pickford et al, 1966a), Poecilia latipinna (Ball and Ensor, 1965,1967), Fundulus kansae (Stanley and Fleming, 1967b), Anguilla anguilla (Butler, 1966; Olivereau and Chartier-Baraduc, 1966; Chan et al,1968), Tilapia mossambica (Dharmamba et al, 1967) and Carassius auratus (Donaldson et al, 1968; Ogawa, 1968; Lahlou and Sawyer, 1969a; Lahlou and Giordan, 1970). Tolerance to freshwater may be related to the rate of decline in plasma sodium which, in the eel, may be very slow with prolonged survival (Olivereau and Fontaine, 1965; Olivereau and Chartier-Baraduc, 1966) or quite rapid with shorter survival (Butler, 1966). Alternatively a high tolerance of the tissues to internal dilution may be important, since the goldfish can survive with a 33% decrease in plasma sodium chloride (Lahlou and Sawyer, 1969a).

Variations in tissue electrolytes other than sodium and chloride following hypophysectomy may be slight or erratic. Butler (1966) noted reductions in plasma potassium, muscle sodium and potassium, and an increase in muscle water in the freshwater eel. Chan et al (1968) confirmed the increase in muscle water but found an increase in serum potassium and a reduction in calcium. In Fundulus kansae serum calcium increased but potassium content remained unaltered (Stanley and Fleming, 1967b).

Isotopic kinetic studies have demonstrated that sodium exchange between teleosts and external freshwater media is impaired by hypophysectomy (Potts and Evans, 1966; Maetz et al, 1967a,b; Ensor and Ball, 1968a). Upon transfer from SW to FW the sodium turnover of intact Fundulus heteroclitus

and Anguilla anguilla is soon reduced from circa 30% hr to < 1% hr (Maetz et al, 1967a,b). On the second day in freshwater Fundulus heteroclitus achieves a positive sodium balance and that in the eel is only very slightly negative. In hypophysectomised Fundulus heteroclitus sodium efflux is twice that in intact fish and sodium balance remains highly negative (net flux -27.8 ± 7.47 μeq/hr/100 gm, n=10). In the eel hypophysectomy does not alter the process of flux readjustment upon transfer. The critical external sodium concentration permitting sodium equilibrium is lower in the freshwater eel (0.1 meq/l) than in the killifish (0.4 meq/l). At this limiting sodium concentration hypophysectomy does increase sodium efflux in the eel resulting in a negative sodium balance (-2.50 ± 0.30 μeq/hr/100 gm n=20) but the net flux is much lower than in the killifish.

Potts and Evans (1966) reached conclusions similar to those of Maetz et al (1967a) concerning the increased rate of sodium depletion in operated Fundulus heteroclitus in freshwater. Ligation of the anal and urinary apertures demonstrated that the major route of sodium loss was extrarenal. This is in agreement with Maetz et al's (1967a) estimates that renal sodium loss is only 1/20 - 1/50 of the branchial efflux soon after transfer, and circa one third in FW-adapted killifish. Lahlou and Sawyer (1969a) analysed sodium fluxes in the goldfish and found a strong negative sodium balance in hypophysectomised fish. Renal sodium loss never exceeded 20% of the total negative net flux and was not increased by pituitary ablation, confirming that the main effect increasing sodium depletion was extrarenal in origin.

Isotopic studies have also shown that hypophysectomised Poecilia latipinna and Tilapia mossambica in freshwater are in strong negative sodium balance with elevated rates of sodium efflux (Ensor and Ball, 1968; Dharmamba, unpublished, 1969).

An increase in sodium efflux, almost certainly across the gill membrane, is the major extrarenal effect of hypophysectomy upon teleosts in freshwater. Maetz et al (1967a,b) observed no alteration in absolute sodium influx in Fundulus heteroclitus. Since sodium uptake is dependent upon the availability of the ion in the external medium influx is better expressed as the ratio $^{f}in/Na_{ext}$. A 50% reduction in the efficiency of branchial sodium absorption was then apparent in hypophysectomised Fundulus heteroclitus. In the eel, however, absolute sodium influx and $^{f}in/Na_{ext}$ showed a 20% enhancement, but a more extensive increase in efflux resulted in a negative net flux. Earlier Butler (1966) had suggested that sodium depletion in ablated eels was due to a reduction in net branchial influx since renal losses of the ion were reduced. Without the use of radioactive isotopes it is not possible to resolve the effects of hypophysectomy upon the influx and efflux components.

Hypophysectomy is also known to influence the renal function of teleosts in freshwater. In Fundulus kansae urine flow is reduced but the osmolality, sodium and potassium content of the urine are increased (Stanley and Fleming, 1966a, 1967b). The reduction in serum sodium in this teleost is due to an impaired tubular reabsorption of sodium that permits an increase in the renal efflux of the ion. The branchial efflux is not affected by hypophysectomy and the influx is

enhanced (Stanley and Fleming, 1966a, 1967b). These authors suggested that the low urine flow reflected a decrease in the permeability and net influx of water. Later Potts and Fleming (1970) provided confirmation since the turnover rate of tritiated water was reduced following hypophysectomy.

The ablation has similar effects upon the flow rate, osmolality and sodium content of the urine in Carassius auratus (Ogawa, 1968; Lahlou and Sawyer, 1969a; Lahlou and Giordan, 1970). Lahlou and Sawyer found that urine flow was 55% of normal levels. The sodium content increased but the renal efflux of the ion remained unaltered, which, considered in relation to reduced plasma sodium, might have represented an increased depletion. Lahlou and Giordan observed a reduction in water permeability but the associated decrease in urine flow was such that body weight increased, suggesting a regulation of urine flow independent of external permeability. In Anguilla anguilla hypophysectomy produced a decline in urine flow and sodium excretion (Chester-Jones et al, 1965; Butler, 1966). Water retention was reflected in the hyperhydration of muscle tissue observed in this teleost (Chan et al, 1968).

Recent investigations upon water balance in various teleosts have evaluated both osmotic permeability (from drinking rate and urine flow), and diffusional permeability using tritiated water (Evans, 1969; Potts et al, 1967; Motais et al, 1969). In Carassius auratus hypophysectomy reduced both the diffusional and osmotic components of net water influx (Lahlou and Sawyer, 1969b; Lahlou and Giordan, 1970). A similar effect upon diffusional permeability was observed in Fundulus kansae (Potts and Fleming, 1970). The results upon the goldfish provided a clear

demonstration that the reduction in plasma electrolytes following hypophysectomy was due to an impaired ionic exchange rather than an increased permeability and consequent influx of water.

In general hypophysectomy in freshwater teleosts leads to an increase in the ionic permeability of the gill membrane which permits a high passive efflux, and hence depletion of sodium. Renal effects upon ionic excretion are variable and of minimal importance, with the exception of Fundulus kansae in which the urine is the sole route of increased sodium depletion.

PROLACTIN

Exogenous Prolactin

In Sea Water

Treatment with mammalian prolactin does not restore the reduced sodium exchange of hypophysectomised Anguilla anguilla and Fundulus heteroclitus to normal levels but ACTH is effective (Maetz et al, 1967a,b,c). Prolactin has no effect upon intact eels but in Fundulus heteroclitus the hormone (1 or 3 injections : 5µg/gm.wt.) reduced sodium turnover from circa 40%/hr to less than 20%/hr for several days (Maetz et al, 1969a;Mayer, 1970). In Tilapia mossambica a high prolactin dose (6 x 10 ug/gm.wt./day) reduced the turnover from 28%/hr to 3%/hr with an increase in plasma sodium to exceed 200 meq/l. The effect is exerted at the gill since the renal sodium efflux is negligible before the branchial ion fluxes. A depression in the net branchial efflux of sodium would account for accumulation of internal sodium.

Stanley and Fleming (1967a) found that prolactin increased serum sodium levels in both intact and hypophysectomised Fundulus kansae held in 0.4M saline. These authors suggested that the hormone increased water permeability rather than reducing sodium efflux, and that osmotic water losses to the hyperosmotic medium led to an elevated sodium content.

In hypophysectomised Poecilia latipinna in one-third sea water the enhanced sodium turnover is restored to normal

levels by chronic prolactin treatment (Ball and Ensor, 1969) With the exception of Fundulus kansae these effects of prolactin correspond to a reduction in branchial sodium permeability.

In Freshwater

Pickford and Phillips (1959) found that injections of ovine prolactin were able to promote the survival of hypophysectomised Fundulus heteroclitus in freshwater. This effect is specific to prolactin. It is not shared by the other pituitary hormones tested except for primate growth hormone which has an intrinsic prolactin activity (Pickford et al, 1965). Since these observations were made numerous reports concerning the beneficial effects of mammalian prolactin upon the survival and osmoregulation of hypophysectomised teleosts in freshwater have been published. Recent reviews of these papers are available (Bern, 1967; Maetz, 1968; Ball, 1969a,b; Olivereau and Ball, 1970).

Prolactin also prolongs freshwater survival in hypophysectomised Poecilia latipinna (Ball and Olivereau, 1964), Gambusia sp. (Chambolle, 1966) and Tilapia mossambica (Dharmamba et al, 1967), Xiphophorus spp. (Schreibman and Kallman, 1964,1966)

The anadromous marine stickleback Gasterosteus aculeatus trachurus exhibits "physiological hypophysectomy" in its inability to survive transfer to freshwater in early winter. Mortality was reduced by prolactin injections (Lam and Leatherland, 1969a).

Data upon the effects of hypophysectomy upon teleosts in freshwater indicate an impairment of the ability to conserve electrolytes. This effect is severe in species that die after

transfer, but remains evident to a lesser extent in those that survive. Treatment with prolactin generally leads to more efficient sodium conservation. Ovine prolactin prevented the reduction in serum osmolality and sodium content in ablated Fundulus heteroclitus in freshwater (Pickford et al, 1966; Pickford et al, 197 and also the fall in plasma sodium and chloride in Poecilia latipinna (Ball and Ensor, 1965,1969). In hypophysectomised Carassius auratus ovine prolactin restored plasma osmolality to normal levels (Donaldson et al, 1968) and partially prevented the decline in plasma sodium and chloride (Lahlou and Sawyer, 1969a). Prolactin treatment reduced the fall in plasma osmolality in operated Tilapia mossambica (Dharmamba et al, 1967) and had a similar effect upon osmolality and plasma sodium and chloride in early winter sticklebacks transfered to freshwater (Lam and Hoar, 1967; Lam, 1968).

In hypophysectomised Anguilla anguilla, which survives in freshwater, the slow decline in plasma sodium, potassium and calcium is delayed by prompt treatment with ovine prolactin though the hormone has no effect upon these plasma ions in intact fish (Olivereau and Chartier-Baraduc, 1966; Olivereau and Olivereau, 1970). In contrast Chan et al (1968) could not correct reductions in plasma sodium and calcium with bovine prolactin but the increase in plasma potassium was restored to normal levels. However in terms of its bioassay activity bovine prolactin is generally less effective than the ovine hormone.

As with the promotion of freshwater survival the action of ovine prolactin upon serum osmolality and electrolytes is specific to this hormone. In Poecilia latipinna oxytocin, vasopressin, arg vasotocin, isotocin, ACTH, GH, TSH and MSH have no effect upon plasma sodium levels (Ball and Ensor, 1967).

Chambolle (1966,1967) noted that ACTH, as well as prolactin, was capable of promoting freshwater survival of hypophysectomised Gambusia sp. and suggested that the two hormones might have a synergistic action in this species.

In hypophysectomised Fundulus kansae prolactin must be administered in high doses before serum sodium is increased (Stanley and Fleming, 1967a; Fleming and Ball unpublished, cited Ball, 1969a); low doses reverse the reduction of urine flow but, despite a reduction in urine sodium, the renal efflux of the ions remains high.

Isotopic kinetic studies have shown that prolactin exerts a major effect upon branchial sodium permeability. In hypophysectomised Fundulus heteroclitus both ovine and bovine prolactin restores the enhanced extrarenal sodium efflux to normal levels (Potts and Evans, 1966; Maetz et al, 1967a). The hormone was injected prior to SW → FW transfer and the prolactin-treated fish attained an equilibrated sodium balance upon the second day in freshwater whilst saline-injected controls remained in negative balance. Sodium influx rates were not affected. Maetz et al (1967b) obtained similar results with Anguilla anguilla. Ovine prolactin reduced the enhanced sodium efflux of hypophysectomised eels to permit a positive sodium balance. The hormone has a similar effect upon the elevated branchial sodium efflux in hypophysectomised Poecilia latipinna (Ensor and Ball, 1968a) and may also limit sodium efflux in the winter marine stickleback (Lam, 1968).

Prolactin does not generally stimulate sodium influx but variable increases in the branchial absorption of sodium occur in hypophysectomised Anguilla anguilla (Maetz et al, 1967b),

Tilapia mossambica (Dharmamba unpublished, 1969), and Fundulus kansae (Fleming and Ball unpublished, cited Ball, 1969a).

Stanley and Fleming (1967b) concluded that the primary effect of prolactin in Fundulus kansae was upon water permeability. They explained the increased urine flow and reduced urine sodium after treatment as a renal response to an increase in net water influx across the external epithelia. In 0.4M saline the high serum sodium was an indirect result of an increase in water permeability allowing a net water loss to the hyperosmotic environment. Recently Potts and Fleming (1970) have confirmed that hypophysectomy reduced water turnover in Fundulus kansae in freshwater ,and that prolactin was capable of restoring the turnover to normal levels. The effects of prolactin upon water permeability are associated with low doses of the hormone and appear to be independent of the enhanced sodium influx in response to high doses (Ball, 1969a).

In Carassius auratus prolactin treatment enhances water exchange leading to tissue hydration. In hypophysectomised fish the hormone corrected a reduction in urine flow and decreased urine sodium content and the renal loss of this ion (Lahlou and Giordan, 1970). In contrast to Fundulus kansae (Stanley and Fleming, 1967b) control of urine flow in the goldfish is independent of external water permeability since hypophysectomy is followed by an increase in weight produced by renal water retension despite a decrease in external permeability (Lahlou and Giordan, 1970). The renal water efflux may be altered by variations in the number of active glomeruli rather than adjustments in tubular

resorption of water (Hickman, 1965; Lahlou, 1966). Bovine prolactin is effective in preventing the hyperhydration of muscle tissue that occurs in the hypophysectomised eel indicating a reduced external water permeability in this species (Chan et al, 1968). This action may be related to the stimulation of mucous cell development that the hormone produces in various teleosts (Egamii and Ishii, 1962; Blüm and Fiedler, 1965; Blüm, 1966; Leatherland and Lam, 1969).

Histological data upon intact and hypophysectomised freshwater eels (Anguilla anguilla) indicate that prolactin treatment stimulates renal function (Olivereau and Lemoine, 1968,1969). Hypophysectomy leads to a reduction in the tubule nuclei and tends to lessen the response to prolactin though cell height and tubule diameter are increased, and new tubules may differentiate. In intact fish the response to hormone treatment is more marked with a general stimulation of the renal tubule, particularly the initial collecting segment. It was suggested that the presence of another hypophysial factor, perhaps ACTH, might elicit the full response in ablated fish. Prolactin treatment might permit a partial restoration of the renal defects associated with hypophysectomy. Chester-Jones et al (1965) and Butler (1966) observed a reduction in flow and an increase in sodium content of the urine of hypophysectomised eels though the renal sodium efflux was reduced slightly. Later Chan et al (1968) showed that simultaneous treatment with prolactin and ACTH was necessary to restore fully defects in the ionic and water balance of ablated eels.

In sticklebacks seasonal differences in endogenous prolactin secretion may regulate changes in the structure and function of the renal corpuscle associated with the requirement of different environments. Exogenous prolactin increases

the GFR and reduces urine osmolality in freshwater (Lam and Hoar, 1967; Lam and Leatherland, 1969b).

Recently Pickford et al (1970b) have found that prolactin controls the activity of the sodium transport enzyme Na + K-ATPase in the osmoregulatory organs of freshwater Fundulus heteroclitus. The hormone decreases the activity of the enzyme in the gill so correlating with the prolactin-induced reduction in branchial sodium efflux produced in hypophysectomised fish. Similarly prolactin increases enzyme activity in the kidney thus favouring sodium resorption and retention.

Endogenous Prolactin

Data from investigations upon survival and osmoregulation of hypophysectomised teleosts in freshwater indicate that the pituitary gland secretes a factor(s) necessary for normal osmoregulation. Successful replacement therapy with mammalian prolactin in many species strongly suggests that this factor is a prolactin-like hormone. Further confirmation is obtained from investigations made upon the teleost pituitary gland. Recent reviews concerning the endogenous "prolactin" of teleosts are available (Maetz, 1968; van Oordt, 1968 ; Ball, 1969a,b; Ball and Baker, 1969; Olivereau and Ball, 1970).

Pituitary brei of the freshwater perch Perca flavescens and of Fundulus heteroclitus maintained the hypophysectomised killifish in freshwater (Burden, 1956). A similar response was obtained with Poecilia latipinna using pituitary homogenates from this species (Ensor and Ball, 1968b). In Poecilia formosa and Poecilia latipinna single ectopic pituitary transplants maintained freshwater survival over long periods (Ball et al, 1965; Ball and Olivereau, 1965; Ball unpublished, cited Olivereau and Ball, 1970). Such

transplants reduced sodium efflux in freshwater and sodium turnover in one-third seawater (Ball and Ensor unpublished, cited Olivereau and Ball, 1970). Acid-acetone extracts of salmon pituitaries maintain plasma osmolality at normal levels in hypophysectomised goldfish in freshwater (Donaldson et al, 1968).

Bioassays for prolactin are based upon the physiological actions of the hormone in higher vertebrates: stimulation of the mammary gland, the pigeon crop-sac, or induction of water-drive in urodele amphibians (Grant and Grant, 1958; Riddle, 1963; Grant and Cooper, 1965; Meites and Nicoll, 1966; Nicoll, 1969). Teleostean pituitary extracts do not exhibit mammotrophic activity (Nicoll et al, 1966) but are partially effective in the pigeon crop-sac assay (Chadwick, 1966;1970; Nicoll et al, 1966; Nicoll and Bern, 1968) and fully active in promoting water-drive in the hypophysectomised red-eft Diemyctilus viridescens (Grant and Pickford, 1959). Nicoll and his co-workers elicited only a minimal response in the pigeon crop-sac assay. However Chadwick (1965,1966) found that extracts induced a thickening and folding of the crop-sac mucosa although the fatty granules in the mucosal epithelium typical of the full response were absent. Later Chadwick (1970) extracted a fraction from flounder pituitaries by polyacrylamide electrophoresis. This fraction gave a positive response in the pigeon crop assay and also had a similar electrophoretic mobility to tetrapod prolactin. Since this teleostean pituitary factor possessed some of the properties of mammalian prolactin but not the full crop-sac activity Ball (1965c) suggested that it be named "paralactin".

Olivereau and Herlant (1960) noted that the erythrosinophilic or eta cells in the rostral pars distalis of the teleostean pituitary had similar staining properties to the mammalian prolactin cell. Emmart et al (1966) found that fluorescent anti-ovine prolactin bound to the eta cells in frozen sections of Fundulus pituitaries. A similar antigenic activity is localized within the eta cells of Oncorhynchus nerka (McKeown and van Overbeeke, 1969) and Carassius auratus (Emmart, 1969). Fluorescent anti-prolactin binds to the cytoplasmic granules of Fundulus heteroclitus eta cells cultured in vitro (Emmart and Mossakowski, 1967). Antibodies raised from teleost pituitary extracts exhibit a cross-reaction with highly purified ovine prolactin (Emmart and Wilhelmi, 1968).

Further evidence that the source of piscine prolactin is located within the eta cells is derived from observations upon pituitary transplants in Poecilia spp. Ectopic transplants promoting freshwater survival in Poecilia latipinna and Poecilia formosa always include active eta cells (Ball and Kallman, 1962; Ball et al, 1965; Ball and Olivereau, 1965; Olivereau and Ball, 1966), but transplants containing few eta cells (proximal pars distalis + neurointermediate lobe) are ineffective in Poecilia latipinna (Ball, 1965c). Surgical removal of the eta cell zone in this species impairs freshwater tolerance (Ball, 1965b) and a regenerated pituitary remnant in an incompletely hypophysectomised fish was composed of eta cells and shown to be secreting a prolactin-like factor (Ball, 1965b).

Ball and Baker (1969) have reviewed the anatomy and histophysiology of the teleost pituitary. Information concerning the prolactin cells is derived mainly from the extensive studies of Olivereau and Ball upon the pituitary

glands of Anguilla anguilla and Poecilia spp. (Ball and Olivereau, 1964, 1965; Olivereau and Ball, 1964, 1966, 1970; Ball, 1969a,b; Olivereau, 1966a, 1967, 1969a; Olivereau and Dimovska, 1968, 1971). In the rostral pars distalis of the eel the eta cells are columnar with ciliated apices and arranged in follicles as in other more primitive teleosts (salmonids, clupeoids). In Poecilia spp. and non-isopendylous teleosts the eta cells dominate the rostral pars distalis but they are non-columnar and without follicular formation (Olivereau and Ball, 1964). The eta granules which are the source of prolactin (Emmart and Mossakowski, 1967) exhibit specific staining properties (erythrosinophilic) and appear upon electron micrographs as dense membrane-bound osmophilic vesicles derived from the endoplasmic reticulum via the Golgi apparatus.

Changes in the activity of the teleost eta cells may be correlated with the salinity of the external environment. These cells are more active in freshwater than in sea water (or dilute sea water) in Poecilia spp. (Ball and Olivereau, 1964; Olivereau and Ball, 1964; Ball, 1969a,b). Fundulus heteroclitus (Ball and Pickford, 1964; Emmart et al, 1966), salmonids (Olivereau, 1954; van Overbeeke and McBride, 1967), eels (Olivereau, 1966a,b), Mugil sp. (Olivereau and Ball, 1964; Abraham et al, 1967; Olivereau, 1968), Fundulus kansae (Ball and Fleming, 1968 cited Ball and Baker, 1969), and Tilapia mossambica (Dharmamba and Nishioska, 1968).

In Tilapia mossambica in freshwater the rostral lobe of the pituitary and its eta cells are enlarged. Active secretion is indicated by the numerous large granules, prominent Golgi apparatus and multilayered endoplasmic reticulum. In marine conditions the rostral lobe is smaller,

the eta cells condensed and granule size and organelles reduced suggesting low synthetic activity (Dharmamba and Nishioka, 1968). Freshwater Fundulus heteroclitus have an enlarged eta cell region exhibiting intense activity with cellular, nuclear and nucleolar hypertrophy, high RNA content, a prominent Golgi apparatus and high mitotic index (Ball and Pickford, 1964). In sea water eels the eta granules are small and less numerous than in freshwater fish (Knowles and Vollrath, 1966b; Olivereau, 1966a).

The results of in vitro studies upon prolactin cells and their response to osmotic stimuli agree with cytological observations upon in situ glands (Sage, 1966). Xiphophorus spp. eta cells lose more acidophilia and weight when cultured upon diluted media than on concentrated medium suggesting a secretory response to dilution (Sage, 1968). Hopkins (1969) found that the secretory mechanism of Poecilia latipinna eta cells was initially stimulated in freshwater then inhibited by salt water. Inhibition was accompanied by an increase in acid phosphatase activity concerned in the removal of surplus secretory products.

The secretory activity of prolactin cells is reduced but not completely inhibited in seawater. A marine environment does not prevent the development of the rostral follicular eta cell zone in salmon and trout (Olivereau, 1969a). Pituitary transplants in Poecilia latipinna are capable of reducing the sodium turnover rate of fish in one-third sea water (Ball and Ensor, 1969; Ball unpublished, cited Olivereau and Ball, 1970).

The stimulus for an increase in endogenous prolactin secretion may be a reduction in internal sodium content

associated with a transfer to hypotonic media. Aldactone-induced hyponatraemia stimulated eta cell activity in the eel (Olivereau and Chartier-Baraduc, 1965).

In teleosts the evidence for a hypothalamic control of prolactin secretion is equivocal. In mammals prolactin secretion is under the inhibitory control of the hypothalamus; severance of hypothalamic connections or biochemical blockage of the prolactin-inhibiting factor by reserpine administration stimulates prolactin production in the pars distalis (Meites and Nicoll, 1966). The similar patterns of activation of intact pituitary glands and ectopic transplants in Poecilia latipinna following one-third SW→FW transfer (Ball and Olivereau, 1965; Ball, 1969a), and the persistence of salinity-dependent alterations in the activity of in vitro cultured Xiphophorus eta cells (Sage, 1966, 1968) indicate that hypothalamic connections are not essential for prolactin secretion but do not exclude the possibility of inhibitory control. Suppression of the hypothalamic connections stimulates the eta cells slightly in Poecilia formosa (Olivereau and Ball, 1965). Long-term prolactin treatment reduces the activity of the prolactin cells in Anguilla anguilla (Olivereau, 1969a). In the eel the effect of reserpine treatment in activating the eta cells is slight (Olivereau, 1967, cited Olivereau, 1969a), but in the goby Gillichthys mirabilis the drug evoked a pigmentary response correlated with increased prolactin secretion, indicating the presence of a hypothalamic prolactin inhibitor in this species (Sage, 1970).

ADRENOCORTICOSTEROIDS AND HYPOPHYSIAL ACTH

Identification of Steroids

In teleosts the adrenocortical tissue (also termed the interrenal gland or suprarenal bodies) is located in the anterior head kidney often in close association with the cardinal veins. The identification and occurrence of adrenocorticosteroid hormones in teleosts have been reviewed recently by Gottfried (1964), Seal and Doe (1965), Nandi, (1967), Chester-Jones et al (1969a) and Henderson et al (1970). A bibliography of relevant literature was prepared by Bern and Chieffi (1968). Cortisol, cortisone and corticosterone have been identified in teleostean plasma (Phillips and Chester-Jones, 1957; Phillips, 1959; Idler et al, 1959a,b; Leloup-Hatey, 1964). Cortisol is the major adrenocorticosteroid present (Bondy et al, 1957; Phillips and Chester-Jones, 1957; Phillips, 1959; Hane and Robertson, 1959; Chester-Jones et al, 1959; Idler et al, 1959b; 1964; Schmidt and Idler, 1962; Leloup-Hatey, 1964; Donaldson and MacBride, 1967; Bradshaw and Fontaine-Bertrand, 1968; Singley and Chavin, 1968) and occurs in quantities in the order of µg/100 ml plasma (Chester-Jones et al, 1969a). In Anguilla spp. plasma cortisol levels are 2-4 µg/100 ml plasma (Butler et al, 1969b; Forster unpublished, cited Henderson et al, 1970).

Incubation of interrenal tissue yields the three major steroids, with cortisol predominating (Nandi and Bern, 1965; Leloup-Hatey, 1966). Aldosterone, the potent mammalian

mineralocorticoid, has been reported in salmon Oncorhynchus nerka plasma in minute amounts, 0.12 µg/100 ml, (Phillips et al, 1959) and also as a product of the in vitro incubation of progesterone with interrenal tissue from Fundulus heteroclitus (Phillips and Mulrow, 1959). The presence of aldosterone in teleosts remained in dispute (Idler et al, 1959; Leloup-Hatey, 1966; Sandor et al, 1966; Nandi, 1967) until this steroid and 18-hydroxycorticosterone were obtained from the in vitro incubation of exogenous corticosterone with Clupea harengus interrenal tissue (Truscott and Idler, 1968). Minute quantities (<1 - 65 ng/100 ml) of aldosterone were later identified in the plasma of the herring (Truscott and Idler, 1969) but its presence and biosynthesis in other teleostean species have yet to be demonstrated. In Anguilla anguilla a low activity 18-hydroxylating system converting exogenous corticosterone to 18-hydroxycorticosterone is present but no aldosterone is produced (Sandor et al, 1969)

Effects upon Osmoregulation

Most investigations upon osmoregulation have concerned Anguilla spp. which have been adrenalectomised successfully (Chester-Jones et al, 1964; Butler and Langford, 1967; Chan et al, 1967; Butler et al, 1969a,b). In SW-adapted Anguilla anguilla adrenalectomy leads to dehydration and increases in plasma sodium, potassium, calcium, magnesium and chloride; in consequence, survival is impaired (Chan et al, 1967). Reductions in body weight and muscle water, and high muscle electrolytes are indicative of an increase in branchial water permeability. The survival of ablated eels is restricted to circa 48 hr in sea water, but is indefinite

in one-third sea water (Chan et al, 1967; Mayer et al, 1967). Mayer et al correlated increases in plasma sodium and chloride with marked reductions in sodium turnover and branchial sodium efflux.

Adrenalectomy delays the readaptation of branchial sodium efflux following the transfer of Anguilla anguilla from freshwater to sea water. Cortisol treatment (in physiological doses) restores plasma sodium to normal levels and prolongs the survival of ablated eels in sea water (Mayer et al, 1967; Mayer and Maetz, 1967; Mayer, 1970). These beneficial effects are mediated by a restoration of a normal branchial sodium excretion. Cortisol injections (3 x 50 µg/100 gm) increase sodium efflux from 500 meq/hr/100 gm to 1500 meq/hr/100 gm. Hypophysectomy has a similar effect to adrenalectomy in depressing the sodium turnover and branchial sodium efflux of SW-adapted eels. The response to pituitary ablation is mediated by a reduction in hypophysial-interrenal stimulation since treatment with exogenous ACTH, cortisol or aldosterone restores sodium exchange to normal levels (Mayer, 1970).

In adrenalectomised Anguilla anguillla maintained in freshwater a retention of water is associated with a reduction in plasma electrolytes. These effects extend to the muscle tissue (Chan et al, 1967). A low external sodium concentration (0.060 meq/1) provokes a further decline in plasma sodium and death follows within one week (Chester-Jones et al, 1965; Chan et al, 1967; Henderson and Chester-Jones, 1967). In ablated Anguilla rostrata exhibiting a low plasma cortisol only plasma magnesium is reduced, but a more extensive removal of the posterior cardinal veins and related adrenocortical tissue has effects upon plasma and muscle electrolytes similar to those seen in the European eel (Butler et al, 1969b). A decrease

in GFR and urine flow accompanied by a normal or enhanced electrolyte excretion in adrenalectomised Anguilla anguilla explains in part the increased water retension (Chan et al, 1969).

In freshwater eels the branchial absorption of sodium (expressed both as absolute influx and $^{f}_{in}/Na_{ext}$) is impaired by adrenalectomy (Henderson and Chester-Jones, 1967; Maetz et al, 1969a; Maetz, 1969a; Mayer, 1970). Sodium efflux at the gill is not affected, being high in both sham-operated and ablated eels. It is probable that surgical lesions of the skin and circulatory system aggravate sodium efflux since the rate is normal in ablated eels with fully-healed incisions (Chan unpublished, cited Mayer, 1970).

High (mg) doses of cortisol increase the net extrarenal sodium efflux of intact eels but physiological doses enhance the net influx of intact and ablated fish (Chester-Jones et al, 1967; Henderson and Chester-Jones, 1967; Chan et al, 1969). Aldosterone treatment may also restore sodium uptake to normal levels (Chester-Jones et al, 1962; Henderson and Chester-Jones, 1967) but only cortisol, and to a lesser extent 11-deoxycorticosterone, are effective in repairing defects in urine flow and electrolyte content (Chan et al, 1969). A reduction in intestinal water permeability following adrenalectomy is reversed by cortisol treatment (Gaitskell unpublished, cited Henderson et al, 1970). Cortisol also promotes the net influx of sodium across the intestine in vitro (Payan, unpublished, cited Maetz, 1970c). This latter effect upon sodium transport can be correlated with the action of cortisol in increasing the intestinal Na + K - ATPase of Fundulus heteroclitus (Pickford et al, 1970a) and Anguilla rostrata (Epstein et al, 1971).

Hypophysial-interrenal axis

The evidence for existence of a hypophysial control of interrenal activity in teleosts has been reviewed recently by Hoar (1966), van Oordt (1968), Maetz (1968,1969a), Fleming (1968), Ball and Baker (1969) and Chester-Jones et al (1969a). Reports concerning the identification and salinity-dependent histophysiology of ACTH cells in the pituitary gland have been reviewed by Ball and Baker (1969) and Olivereau and Ball (1970).

Hypophysectomy of Anguilla anguilla and various other teleosts is followed by a reduction in interrenal activity (Fontaine and Hatey, 1953; Olivereau and Fromentin, 1954; Olivereau, 1965; Leloup-Hatey, 1964, 1968). Adrenalectomy or pharmacological inhibition of steroidogenesis and steroid activity permits the localization of adrenocorticotropic activity in the epsilon cells of the rostral pars distalis of the pituitary gland, and produces a feedback hypertrophy and degranulation of these cells (Ball and Olivereau, 1966; Hanke et al, 1967; Fagerlund et al, 1968; Olivereau, 1965, 1968). Using a fluorescent antibody technique McKeown and Overbeeke (1969) located ACTH activity in the epsilon cells of Oncorhynchus nerka. Anti-porcine ACTH bound specifically to these cells.

In the eel hypophysectomy depresses the hypertrophic and hyperplastic responses of the interrenal tissue to pharmacological inhibition (Olivereau, 1965). Interrenal atrophy in hypophysectomised Couesius plumbeus is reversed by injection of salmon pituitary extracts; mammalian ACTH is less effective (van Overbeeke and Ahsan, 1966). Total or partial adrenalectomy stimulates ACTH activity with a marked effect upon

adrenal tissue remnants (Olivereau, 1968; Hanke et al, 1967; Olivereau and Olivereau, 1968).

Injections of mammalian ACTH induce a hypertrophy of the adrenocortical tissue of teleosts (Rasquin, 1951; Burden, 1956; Chavin, 1956; Pickford and Atz, 1957; Basu et al, 1965; Hanke and Chester-Jones, 1966; Hanke et al, 1967; Fagerlund et al, 1968). Conversely injections of cortisol are followed by a regression of both ACTH cells and interrenal tissue, indicating a feedback inhibition of endogenous steroid secretion (Olivereau, 1966b; Hanke and Chester-Jones, 1966; Hanke et al; 1967).

The hypophysial-interrenal axis has also been investigated by direct measurements upon plasma steroids. Plasma cortisol levels are reduced by hypophysectomy in Anguilla anguilla and Anguilla rostrata, and can be partially restored by treatment with teleostean pituitary extracts or mammalian ACTH (Leloup-Hatey, 1964, 1968; Bradshaw and Fontaine-Bertrand, 1968; Butler et al, 1969a,b). In Salmo gairdnerii plasma cortisol is reduced from 8.1 to 3.6 µg/100 ml by pituitary ablation. Dexamethasone, a synthetic steroid, reduces plasma cortisol in both the latter species and Oncorhynchus nerka through feedback inhibition mediated via the pituitary gland. Dexamethasone does not act directly upon the interrenal gland since exogenous ACTH remains capable of promoting cortisol production (Donaldson and McBride, 1969; Fagerlund et al, 1968; Fagerlund and McBride, 1969).

Maetz (1968,1969a) suggested that the stimuli for alterations in hypophysial-interrenal activity would be salt-loading in sea water and salt depletion in freshwater. Salt-depleted teleosts in deionized water compensate by increasing branchial sodium absorption (Krogh, 1939, Maetz,

1964; Garcia Romeu and Maetz, 1964; Henderson and Chester-Jones, 1967; Favre unpublished, cited Olivereau and Ball, 1970). Salt-loaded Carassius auratus in isotonic saline decrease branchial ion uptake (Garcia Romeu and Maetz, 1964). Such effects might be mediated by changes in ACTH secretion and interrenal activity. Endogenous corticosteroids may influence sodium fluxes by controlling the synthesis of branchial Na + K-ATPase, which is associated with sodium transport activity. Cortisol increases the branchial Na + K - ATPase activity of hypophysectomised Fundulus heteroclitus in sea water (Pickford et al, 1970a), and fully restores the reduced branchial enzyme activity of ablated Anguilla anguilla (Milne et al, 1971).

CHAPTER III

MATERIALS AND METHODS

Supply and Maintenance of Flounders

In Stirling

The flounders used in the experiments at Stirling were obtained from the River Forth at Kincardine Bridge, and also from the River Ythan, Aberdeenshire. Fish from the Forth were caught by boom net fishing of the flood and ebb tides. On the Ythan estuary the rising tide was netted with a beach seine, or flounders were caught by line and hand-net. The fish were transported to Stirling in sea water under oxygen. On the longer journey from Aberdeenshire the fish were cooled by ice.

The flounders (80-250 gm) were maintained in a marine aquarium consisting of eight shallow fibreglass tanks. The total capacity was circa 600 litres and the sea water was continuously recirculated through a sand filter. Some aeration did occur during circulation but this was supplemented by four Hyflo pumps. The aquarium was in a constant temperature room ($10 \pm 1°C$) with a 12 hr light/dark regime. The salinity was maintained at 34.5% with frequent additions of copper-free fresh-water to compensate for evaporative losses. At weekly

intervals *circa* 100 litres of water was withdrawn to be replaced with fresh sea water.

The flounders usually began to feed during their first week in the aquarium. They were provided *ad libitum* with minced squid and limpet. Some fish, obviously abstaining from food, were force fed by the injection of homogenised squid directly into the stomach via a soft, plastic tube. The flounders adapted well to the aquaria and generally proved to be hardy experimental animals. Some senestral flounders (which were not hypophysectomised) survived for longer than six months, but spent fish caught near the end of the breeding season (April-May) often showed a more limited (one month) survival period. Disease presented no problems in the aquaria; fish suffering from acute lymphocystis (a pathogen common in *Pleuronectidae*) had been discarded at the time of catching.

A freshwater aquarium was installed in the same constant temperature room. This was a single fibreglass tank of capacity *circa* 250 litres. A continuous flow of "copper-free" water (piped in plastic from cast-iron mains) passed through a coiled heat exchanger in the marine aquarium to supply the freshwater system. Hyflo pumps aerated this aquarium.

In Villefranche-sur-Mer

The marine aquaria of the Station Zoologique contained a stock of flounders *Platichthys flesus*. These fish (80-300 gm) had been collected from the estuary of the River Loire on the Atlantic coast of France and flown to

Villefranche during the autumn. The flounders were maintained in Meditteranean sea water (37‰) and fed upon minced mussels. Water temperatures varied with season (13-22°C). During the experiments the fish were transfered to smaller aquaria (SW, 1/3 SW and FW) and were adapted to a temperature of 16 ± 1°C.

Hypophysectomy

Barr (1963) described a technique for hypophysectomy of the plaice Pleuronectes platessa. Subsequently this technique has been modified by Wardle (pers. comm.). The operation was further modified and adapted for use upon small specimens of P. flesus.

Fish of both sexes (80-250 gm) were selected from the marine aquarium. Only the more common dextral variety was used so that operculum was on the right side of the eyes. Each flounder was anaesthetized with MS222-Sandoz (1 gm + 5 litres SW) and at intervals during surgery the gills were irrigated with oxygenated anaesthetic solution.

The flounder was placed upon a flat surface covered with wet tissues and inclined (30°) downwards from the operator. The eyes and skin surface posterior to the operculum were covered with damp tissue. The gills and operculum were retracted in opposite directions to expose the roof of the buccal cavity. A 5 mm incision was made in the mucous membrane parallel and posterior to the transverse ridge on the right side of the midline and anterior to the right pseudobranch. The incision gaped under the retraction to expose the underlying muscle. The incision

was deepened and the pro-otic bone was exposed by removal of extraneous muscle tissue. The braincase was drilled with a 2 mm sclerectomy trephine (Weiss). The drilling position was selected whilst the lateral surface of the braincase was illuminated by an intense light source positioned above the operculum. Transmitted light revealed anatomical structures below the bone. Beneath the pro-otic bone the carotid artery passes to the anterior. The bone was drilled from a vertical aspect superior to this vessel within a triangle formed by the carotid, jugular and trigemino-facial foramena.

The envelope of connective tissue around the brain was perforated and the slit was enlarged with a blunt glass probe. The pituitary gland lay beneath the drill-hole immediately anterior to the saccus vasculosus. The gland was sucked out with a Pasteur pipette. The disc of bone was replaced and the incision sutured with silk using curved, corneal needles (Weiss). The wound had healed completely within two weeks of the operation.

A more direct approach to the gland *via* the base of the braincase was complicated by the thick, ridged parasphenoid bone and intervening blood vessels. If the carotid artery was damaged the braincase filled with blood and further surgery became impossible. Care was required in selection of the drilling position and insertion of the pipette.

The success of hypophysectomy was confirmed by immediate examination of the excised gland and later by a post-mortem, microscopic investigation of the brain.

In the sham-operation (one form of control) the

technique was similar except that no suction was applied to the pipette. The pituitary gland remained *in situ*. Flounders were identified by a series of coded perforations in the anal fin. These were made with a leather punch.

Sampling Techniques

Blood

A sample of blood (0.3 ml) was withdrawn from the caudal vein using a 1 ml disposable syringe and 25 G hypodermic needle. The sample was transfered immediately into 60 µl microhaematocrit tubes charged with 10 IU lithium heparin (100 IU/mg., Evans Medical). These tubes were centrifuged for five minutes at 12,000 G upon a microhaematocrit centrifuge (Hawkesley). The haematocrit was noted and the red cells and first 1 mm of plasma were discarded. The remaining plasma was transfered into small polythene sample tubes which were sealed and stored at -20°C to await analysis. Lithium heparin rather than the sodium salt was used to avoid possible interference with the accuracy of subsequent sodium analyses. The heparin contained less than 1% sodium ,and less than 0.05% potassium and calcium.

A single non-heparinised microtube of blood was spun down and the resulting serum was stored at -20°C under liquid paraffin. This serum was used for determination of osmolality by freezing point depression.

Urine

The flounder possesses a well-defined urinary bladder that lies against the posterior wall of the coelomic cavity

Urine is voided through a papilla superior and slightly posterior to the anus.

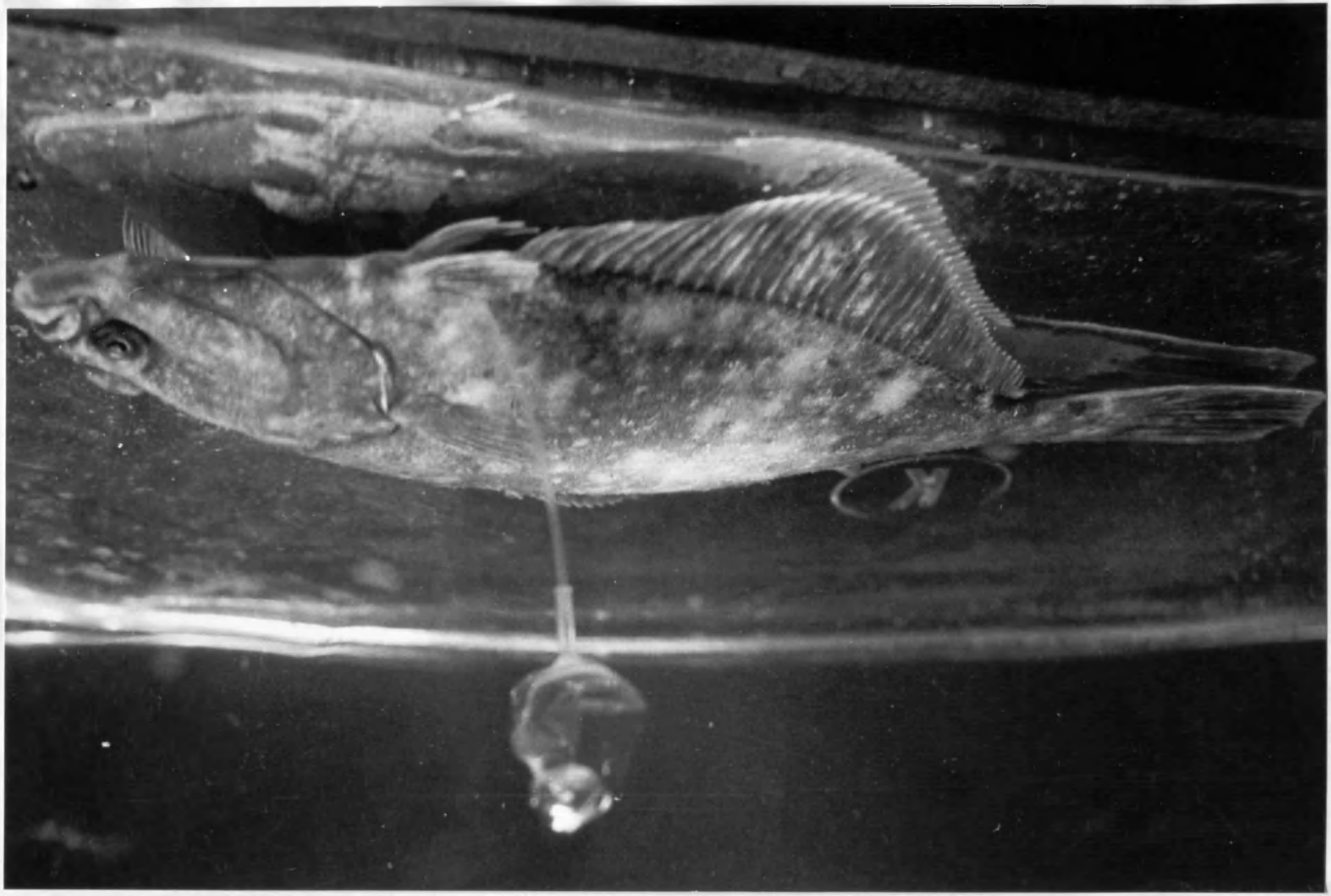
In the first experiment a urine sample was taken at the same time as the blood sample. A short length of PP 60 polythene tubing (Portex) was inserted through the urinary papilla and passed into the bladder. This action frequently stimulated bladder contraction but otherwise gentle pressure was applied to the abdominal wall in the region of the bladder. The urine (10-20 µl) expelled was collected and stored under liquid paraffin in a polythene tube at -20°C.

In the second experiment urine flow was measured and so a permanent catheter was inserted into the bladder of an anaesthetized flounder. The catheter (PP 60) was secured by a double silk suture through the skin posterior to the papilla. A slight swelling (produced over a micro-bunsen) in the catheter just inside the urinary duct prevented leakage of urine. After a period of three or four days in which to check that urine flowed freely a small polythene balloon of capacity 2-5 ml was fixed to the free end of the catheter. The balloon was blown from PP 120tubing over an electric bunsen. Urine was collected over a period 24-48 hr., during which the fish swam freely (Plate 1). Upon removal the balloon was checked for leaks and the quantity of urine determined by weighing. A sample (circa 0.3 ml) was stored at -20°C for analysis.

A series of tests with sodium chloride solutions (Na^+ analysis) showed that the thin polythene membrane was essentially impermeable to water and ions, but wherever possible a small sample of urine was taken directly from

Plate 1.

A flounder fitted with a bladder catheter and polythene balloon during the course of a urine collection.



the catheter for comparison with the main sample. The osmolalities and sodium content of the two types of sample were found to be similar.

Analytical Techniques

Serum and urine osmolalities

The osmotic pressure and freezing point of a solution are both dependent upon the number of dissolved particles present in that solution. The relationship existing between the colligative properties of solutions was employed to determine the osmotic pressures of serum and urine samples from their freezing points. Osmotic pressure is conveniently expressed as osmolality (O) and is calculated from the freezing point depression ($\triangle$) in the equation

$$O = \frac{\triangle \times 1000}{1.858}$$

(O - mOsm/kg water
$\triangle$ - °C)

This equation is based upon the assumption that the addition of 1 mol of non electrolyte to 1 kg of water results in a solution the freezing of which is depressed 1.858°C (Wolf, 1966). In fact the molal freezing point "constant" varies with different solutes and the relationship between freezing points and molality is not strictly linear but O represents the "real" rather than "ideal" osmolality (Wolf, 1966). Its use is perfectly legitimate and well suited to a study in which the comparison of osmolalities was of importance.

The method used to determine the freezing points of blood and urine was based upon that of Ramsay and Brown (1955). Direct measurement of the freezing point is complicated by the problems of supercooling therefore the sample is initially frozen, then allowed to warm slowly and the melting point noted at the disappearance of the last ice crystal (Drucker and Schreiner, cited Ramsay and Brown, 1955).

The modified freezing point apparatus consisted of a freezing compartment containing 70% alcohol and insulated with expanded polystyrene. The controls for mains current, heater and stirrer were housed in a separate unit to avoid heat transference. The alcohol chamber was cooled by the stainless steel freon probe of a refrigeration unit (Type TK1, Rheinische Gerätebau GMBH, Switzerland). The rate of cooling was controlled by a valve that regulated the flow of freon coolant through the probe. The heat supply from the Nichrome element was controlled by two high amperage rheostats that gave a coarse and fine adjustment. Voltage across the element was monitored by a voltmeter. A three-way switch in the circuit allowed fast and slow heating or cooling.

The sample carriage was similar in design to that of the original method. The vitreosil capillaries were cleaned in chromic acid before being drawn out in an oxygen-coal gas flame whereupon they were given a final rinse with hot ethanol. The sample size was of the order 10^{-3}-10^{-4} mm^3.

The glass thermometer was replaced by an integrating quartz thermometer (Hewlett Packard HP2801A) with an

HP2850A probe inserted into the freezing compartment. The temperature was given as a digital display and recorded upon a digital print-out. Maximum resolution was 0.0001°C with a minimum interval of ten seconds. During an $\triangle$ determination the rates of heating and cooling were regulated to give an even rise in temperature of circa 0.0003°C/10 sec.

The quartz thermometer operates on the principle that the resonant frequency of the quartz crystal (in the probe) is sensitive to temperature change. An excellent linear frequency/temperature relationship is attained. The resolution linearity and stability are high providing that the simple precaution of periodic calibration against zero-drift is taken. The instrument was calibrated to an ice-point of 0.0000°C $\pm$ 0.0003°C with an ice bath of crushed ice and water (prepared from deionized water distilled from potassium permanganate solution). The ice-point was corrected for barometric pressure. The final accuracy of the complete procedure for a freezing point determination that could be attained was $\pm$ 0.001°C or $\pm$ 0.5 mOsm/kg.

Plasma and urine electrolytes

Metallic cations The concentrations of sodium, potassium, calcium and magnesium in plasma and urine samples were determined by flame spectrophotometry. The instrument was a Zeiss PMQII spectrophotometer fitted with an MM12 single monochromator and FA2 burner. A Servoscribe analogue recorder was coupled to the galvanometer. Sodium potassium and calcium were analysed by emission and

magnesium by atomic absorption.

Plasma samples (circa 150 µl) were analysed using the standard solutions and diluents (Merck, Titrisol) recommended by Zeiss for use with human serum. The ionic composition of the principal calibration solution was similar to that of fish plasma. The diluent solutions differed depending upon the particular element determined and also served as a blank (except for calcium). All solutions were prepared with deionized water (conductivity < 1 mho). Plasma and urine samples were allowed to attain room temperature from -20°C and the dilutions were prepared with 10 µl and 25 µl Hamilton syringes fitted with Chaney adaptors and Green Line pipettes (E-Mil). Pyrex beakers (10 ml) cleaned in chromosulphuric acid contained the diluted samples. These were sealed with Parafilm prior to analysis. The accuracy of the ion determinations was checked against a freeze-dried control serum (Wellcome) for which precise assay results were available.

Plasma sodium was determined from 5 µl aliquots of plasma diluted (1-401) with 2 ml N/10 hydrochloric acid plus caesium (1 gm CsCl/litre) and wetting agent. The alkali metals have an $\int$-shaped calibration curve with a positive curvature at low concentrations due to the ionization of atoms and negative at high levels due to self absorption (Dean, 1960). The linearity of the calibration curve was improved by the addition of highly ionized caesium that buffered sodium-potassium interference and also by the use of a low temperature flame (hydrogen-air). Caesium also suppressed sodium-potassium ionization.

A high dilution ensured that the physical characteristics of the sample and the calibrant were similar. For sodium a wavelength of 589 mμ and a slit of 0.05 mm were employed. Analysis of the control serum gave 128.5 meq Na/litre against an assay value of 128 meq Na/litre.

Standard methods of potassium analysis required a dilution of circa 1-50 necessitating 40 μl plasma for a final sample of 2 ml. The method of Maybank (1970) required only 5 μl plasma. The method used an organo-aqueous diluent that increased emission and doubled the efficiency of sample consumption such that a 1-200 dilution was possible. A 5μl aliquot of plasma was diluted 1-201 with a caesium/0.1 N-HCl solution plus 60% propan-2-ol by volume. A wavelength of 768 mμ and a slit of 0.14 mm were used in conjunction with a hydrogen-air flame. The calibration curve was linear and the control serum analysis was within 1% of the assay value.

Special blank and calibration solutions were necessary for calcium and magnesium determinations. The blank contained Na and K in the same concentrations as the calibrant to establish the background radiation. The presence of 8-hydroxy-quinoline in the diluent solution (1% acetic acetic acid) prevented the formation of calcium pyrophosphates that would have been difficult to vaporise. Calcium buffered the phosphate during magnesium analysis but the 8-OH-quinoline reduced interference from plasma proteins (as did the high dilution 1-50).

Plasma calcium and magnesium were determined from the same sample containing 60 μl plasma and 3 ml diluent

solution. For calcium a wavelength of 422.7 mμ and a slit width of 0.01 mm were used in analysis by emission. Magnesium was determined by atomic absorption with a hollow cathode lamp emitting at 285.2 mμ. Slit width on the monochromator was 0.05 mm. For both elements the calibration curves were linear and results for the control serum were within 2% of the assay values.

Similar procedures were employed in the cation analyses of urine samples. Where sufficient urine was available a common sample of 40 μl urine plus 5 ml aqueous diluent (1-126) was used. At this dilution sodium levels were determined from a curved calibration graph; the potassium graph remained linear. Magnesium in urine (0.8 - 28.0 meq/l) was often considerably higher than the level in the calibration solution (1.64 meq/l). A special calibration solution with added magnesium was prepared for this analysis, and also to check for possible interferences of magnesium with those other elements determined.

Anions - chloride The chloride content of plasma and urine samples was determined with a Buchler-Cotlove Standard Chloridometer. Cl^- ions in the test solution are precipitated by the release of Ag^+ ions from the generator electrodes. When precipitation is complete the increasing concentration of Ag^+ ions in solution permits an increased current to flow which marks the end point. The "pre-set shut-off" is amperometric and since the rate of supply of Ag^+ ions is constant the Cl^- content is proportional to the titration time giving a linear calibration

line. 10 µl samples of plasma and urine were used, to which were added 2 ml nitric acid reagent (10% CH_3COOH in N/10 HNO_3) and four drops of the gelatin reagent. Analyses of the control serum were accurate to within 1%.

Isotopic Kinetic Studies

Aquaria for radioisotopic techniques

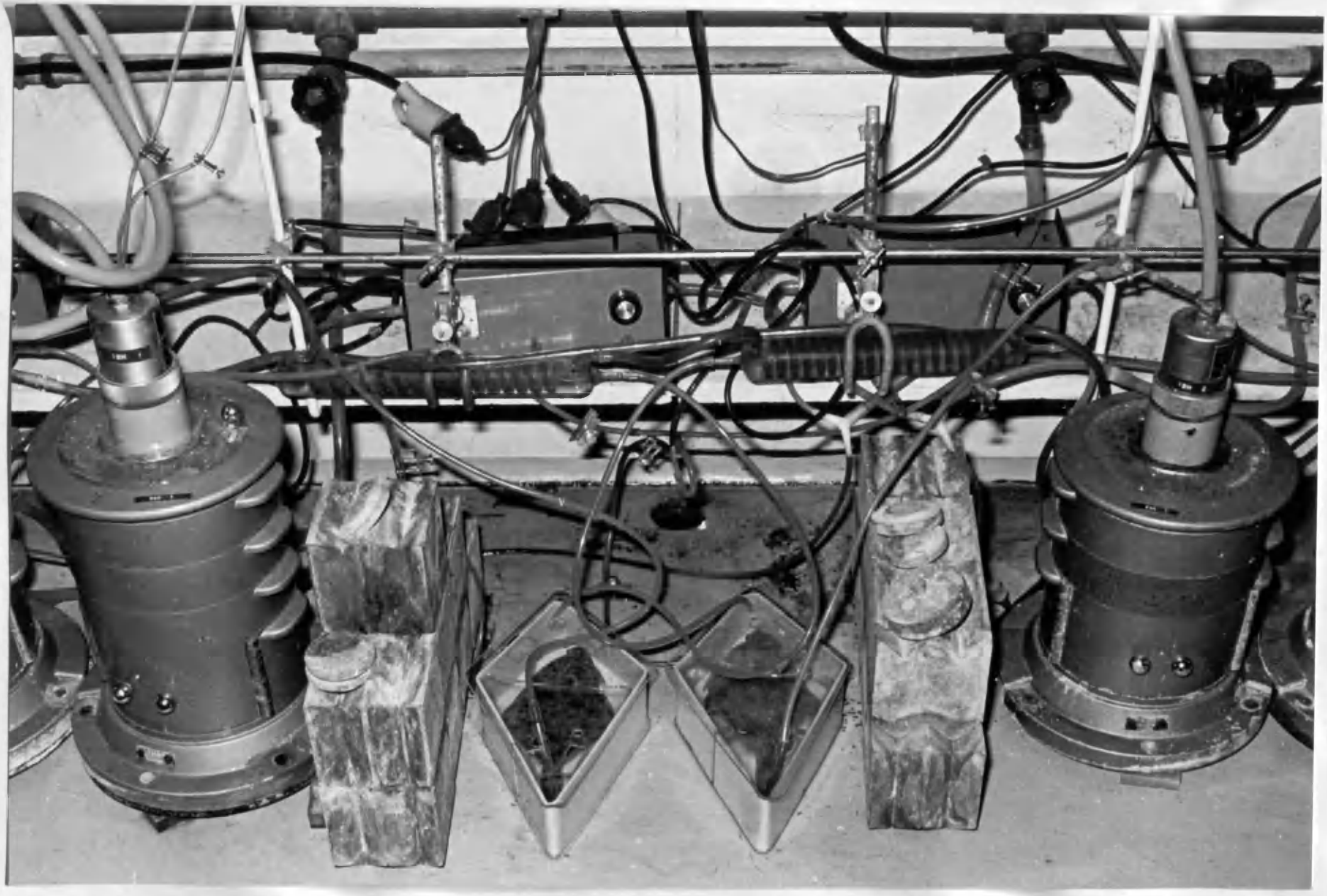
The experimental aquaria in the Station Zoologique were diamond-shaped and 2-3 litres in capacity; their close-fitting design restricted the violent mobility of the fish and required only small volumes of the external medium. A continuous flow of sea water or freshwater was supplied to each aquarium but this could be closed off so that a constant volume of water was recirculted over each fish. Each circuit included a pump, a heat exchanger coil and a spiral, perspex cell situated beneath a β- or γ-scintillation detector shielded within a lead castle (Plate 2). Ten such circuits were available so simultaneous experiments upon the same number of fish were possible. Water temperature was maintained at 16 ± 1°C.

Sodium turnovers in sea water and one-third sea water

Radiosodium ^{24}Na of specific activity 50-250 mC/gm was supplied as sodium carbonate by the Departement des Radioelements, Saclay, France. Doses in the order of 25 µc/100 gm body weight were injected into the intraperitoneal cavity as 0.8% sodium chloride for turnover and efflux measurements in sea water and one-third sea water.

Plate 2.

Twin circuits for the detection of ^{24}Na, each including an aquarium containing a flounder, a water pump, an heat exchanger coil, and a β- or γ-scintillation detector within a lead castle.



In a sodium turnover measurement the injected flounder was placed in the experimental aquarium with running sea water. After allowing 0.5 hr interval for the homogenous distribution of the isotope within the extracellular sodium space of the fish, the circuit was closed and 700 ml of fresh sea water was added. The external medium was circulated through the β- or γ-scintillation detector. External radioactivity was recorded at 5 min. intervals by a digital counter attached to a print-out. The results were automatically corrected for background and decay. Each measurement was normally continued for an eight-hour period, (Maetz & Garcia Romeu, 1964; Maetz, 1964; Motais, 1967; Tanguy, 1970).

External radioactivity increases rapidly during the first few hours (fast component) and then the rate of increase declines (slow component). This pattern of increase corresponds to a three compartment system in parallel and steady state (unidirectional fluxes equal). It is described by the sum of the exponential functions in the following equation (Solomon, 1960; Maetz, 1964; Motais, Garcia Romeu and Maetz, 1966; Motais, 1967):

$$Q = Q_1 (1-e^{-\lambda_1 t}) + Q_2 (1-e^{-\lambda_2 t})$$

Q is the quantity of radioactivity in the external compartment whilst Q_1 and Q_2 are the contributions of the second and third compartments (or fish) to the external radiosodium at equilibrium. The second compartment is the rapidly exchangeable sodium pool in the extracellular space of the fish and λ_1 is its turnover rate with external sodium responsible for the fast component. The third compartment

that produces the slow component is assumed to be the internal or intracellular sodium compartment.

This investigation was concerned with the exchange of extracellular sodium with external sodium and so only λ_1 was required. The fast component dominates the first few hours of a short term measurement (circa 7 hr) and is calculated by analysis of the initial part of the curve (Fig 1). The rate constant λ_2 of the intracellular sodium exchange is small, less important and measurements of a longer duration are necessary for its determination. In effect then the calculations are made for a two compartment system.

$$\lambda_1 = \left(\frac{1}{a_1 V_1} + \frac{1}{a_2 V_2} \right) f_{out}$$

where a_1 and a_2 are the concentrations of stable sodium in the extracellular and external compartments, while V_1 and V_2 are their respective volumes. f_{out} is the rate of sodium efflux from the fish to the external medium (later determined directly). In steady state the sodium pools a_1V_1 and a_2V_2 remain constant. The external pool a_2V_2 is very large compared with a_1V_1 (approximately 2%) therefore $\frac{1}{a_2V_2}$ is negligible before $\frac{1}{a_1V_1}$ and

$$\lambda_1 = \frac{f_{out}}{a_1V_1}$$

The rate constant λ_1 (or λ_{Na}) represents the fraction of extracellular sodium exchanged per unit time and is calculated by graphical analysis of the curve. The evolution of external radioactivity $\frac{\Delta Q}{\Delta t}$ (Δt = 2hr) is

measured at 15 min. intervals and the logarithm of this value is plotted as a function of time. λ_1 is determined from the slope of this plot and expressed as % exchangeable Na/hr.

In one-third sea water λ_1 is measured by a slightly different method as the sodium exchange rate is much slower and the $\frac{\Delta Q}{\Delta t}$ method becomes impracticable. An exact quantity of radiosodium was injected into the flounder which was then immersed in the aquarium containing a measured volume of water. External radioactivity was monitored for a similar period. Upon completion an aliquot of injected ^{24}Na in a similar volume of one-third sea water that corresponded to the theoretical radioactivity at equilibrium was passed through the counter.

Considering this system as being of two compartments only external radiosodium varies with an exponential function of the form

$$Q = Qeq\ (1-e^{-\lambda_1 t})$$

where Qeq is the radioactivity at equilibrium obtained from the aliquot and corrected to include the extracellular sodium space of the fish. The function (Qeq-Q) is plotted as a logarithm against time and the slope represents the equation

$$\log\ (Qeq-Q) = -\lambda_1 t + \log\ Qeq$$

from which the value of λ_1 is obtained.

Extracellular sodium space and sodium efflux in sea water

An accurate dose of radiosodium (in the order of 25 μc/100 gm body weight) was injected intraperitoneally into the flounder and the fish was placed in 700 ml of sea water, (Vext). Samples of 1.0 ml were taken from the external medium at 0.5 hr and 1.5 hr after the injection. At 1.5 hr a blood sample was withdrawn from the candal vein of the flounder. The radioactivities of the external samples (Q_1 and Q_2) and that of aliquots of the injected ^{24}Na (A_o) and plasma (R_1) were determined with a well-counter. The stable sodium concentration (Na_{int}) of the plasma was measured by emission with an Eppendorf flame photometer. The initial interval of 0.5 hr between the injection and sampling permitted homogeneous distribution of the isotope throughout the extracellular sodium space (E). The value of E was obtained from division of the total loss of ^{24}Na by the radioactivity of the plasma aliquot. Thus:

$$E = \frac{(A_o - Q_2)\ Vext}{R} \times \frac{100}{W}$$

where W is the weight of the fish.

The final specific radioactivity of the plasma (SRA_f) is given by the equation

$$SRA_f = \frac{R_1}{Na_{int}}$$

and the change in this value during the 1.0 hr interval is given by

$$\triangle SRA = \frac{(Q_2 - Q_1)\ V_{ext}}{W \times E \times Na_{int}}$$

The median specific radioactivity of the plasma ($\overline{SRA}$) is obtained from

$$\overline{SRA} = SRA_f + \frac{\triangle SRA}{2}$$

The rate of sodium efflux per hour per unit weight (f_{out}) may be calculated from

$$f_{out} = \frac{(Q_2 - Q_1)\ V_{ext}}{\overline{SRA} \times W}$$

Rapid transfer experiments

The instantaneous and delayed regulation of branchial sodium permeability (Motais et al, 1965, 1966) and the Na:K branchial exchange pump (Maetz, 1969b) were studied by rapidly transferring flounders between various external media (e.g. SW→FW→SW). Under such conditions the system fish-external medium was no longer in steady state and so λ Na was not measured. Instead the ratio between effluxes before and after transfer was calculated from the different slopes of the appearance of external radiosodium (Motais et al, 1966; Motais, 1967). Assuming that efflux remained constant a slight decline in plasma specific radioactivity during the course of the experiment was corrected by taking the mean of the slopes of an initial and final period in sea water.

In one experiment (Fig 2) ^{24}Na was injected into the fish and external radiosodium was recorded at 1.5 min. intervals during four successive periods (SW→FW→FW + 10mM K^+ ion→SW) of ten minutes each. The external volume was the same (700 ml) in each medium and at transfer the fish was

rinsed briefly in the new medium. The ratio of the sodium efflux in freshwater and that in freshwater plus potassium (10 mM K^+) to the mean efflux in sea water was calculated from the slopes of the curves. Results were expressed as percentages. Absolute effluxes (μeq/hr/100 gm) were calculated using the results for sodium efflux in sea water previously determined.

In a second experiment (Fig 3) the time course of the regulation of sodium efflux in response to alteration in external salinity was studied. Following ^{24}Na injection flounders were placed in sea water (45 mins), transfered to freshwater (90 mins) and returned to sea water (150 mins). External radioactivity was monitored at 5 min intervals. Plasma samples were withdrawn prior to and upon completion of the measurement. These samples, together with an aliquot of the injected radioactivity, and samples of the external medium at the end of each period were counted upon a well-counter. Plasma sodium content was also determined.

The median specific radioactivity ($\overline{SRA}$) for each period could be calculated since $\triangle$SRA was determined from the accumulation of external radioactivity during each period. With these two values the mean efflux for each period could be calculated. The changing pattern of sodium efflux within each period was deduced from alterations in the slope of the external ^{24}Na trace. The FW-period was divided into an initial 0.5 hr followed by 1.0 hr. From the slope the proportions of external ^{24}Na appearing in the two sub-periods were deduced, and thence the $\overline{SRA}$, and component efflux for each sub-period. The final SW-period was subdivided equally and the separate effluxes for each sub-period calculated.

Sodium fluxes in freshwater

Previous measurements in sea water and one-third sea water were made on the assumption that the flounder was adapted to the external medium and that a steady state (unidirectional fluxes equal) existed between the sodium content of the compartments. In fact a fish under experimental conditions is invariably in a state of sodium depletion or gain such that there is a net flux between it and the external medium; this may be aggravated by shock (Meyer, 1948; Maetz, 1956). Thus far it has been legitimate to ignore this net flux since it is negligible compared with the large sodium content of the compartments considered. However in fresh water the fish sodium compartment (c. 140 meq/l) is very large by comparison with that of the external medium (circa 0.2 meq/l). Under these conditions the net flux of sodium assumes great importance in relation to the low external sodium and leads to extreme complexity in any analysis of fluxes using exponential functions. Therefore the fluxes were determined directly by Maetz's (1956) method.

The influx and net flux of sodium are determined simultaneously as a function of time from evolution curves of $^{24}Na_{ext}$ and total Na_{ext}. Influx is obtained from the formula:

$$f_{in} = -\frac{\Delta Q}{\Delta t} \Big/ \frac{Q}{Na_{ext}}$$

where $\frac{\Delta Q}{\Delta t}$ represents the change in external radioactivity against time and $\frac{Q}{Na_{ext}}$ the specific radioactivity of

external sodium. Net flux is obtained from the formula:

$$f_{net} = \frac{-\triangle Na_{ext}}{\triangle t}$$

and the efflux from the relationship:

$$f_{out} = f_{net} - f_{in}$$

In practice the radiosodium was added to the freshwater in the closed circuit aquarium with minimal disturbance to the fish. External radioactivity was monitored continuously over seven hours. At 0.5 hr intervals 4 ml samples of the external medium were withdrawn with a constriction pipette (Pedersen) for analysis of total sodium content. Injection of the fish was therefore unnecessary and the external volume was measured at the end of the experiment. Trial calculations showed that reverse flux of external radioactivity arising from the fish was minimal and could be ignored in determination of the fluxes.

Tritiated water turnovers

The diffusional water permeability of flounders in sea water was investigated. Turnover rates (λ_{HTO}) of water between the fish and the external medium were determined using tritiated water (HTO). Motais et al (1969) have shown that the branchial epithelium is the main site of HTO efflux.

After intraperitoneal injection of 20 µl of water labelled with tritium (circa 40 mC/ml) the flounder was placed in a closed circuit aquarium with 700 ml of external water. Samples of the external medium (250 µl)

were withdrawn at 15 min or 20 min intervals over eight hours. Further samples were taken from an aliquot of the injected HTO in 700 ml external medium. All samples were added to 5 ml Bray's solution (plus 50 µl distilled water) and counted upon a Nuclear Chicago (Mk. 1) liquid scintillation counter.

The curve for the cumulative appearance of tritium in the external medium (Fig 7) is described by the exponential function in the equation:

$$Q = Qeq\ (1-e^{-\lambda_{H_2O}.t})$$

where Q is the quantity (d.p.m.) of HTO in the external compartment at time t, Qeq is the quantity at equilibrium and λ_{H_2O} the turnover rate (Motais, 1967; Motais et al, 1969). The constant is deduced from the slope of a semilogarithmic plot of (Qeq-Q) against time, given that:

$$\lambda_{H_2O} = \left(\frac{1}{V_1} + \frac{1}{V_2}\right) f_{out}$$

where V_1 and V_2 are internal and external water pools respectively. λ_{H_2O} is corrected to allow for the water space of the fish, for which the value 70% of the body weight has been taken (Thorson, 1961; Lahlou and Sawyer, 1969b).

CHAPTER IV

RESULTS

EFFECTS OF HYPOPHYSECTOMY ON BLOOD COMPOSITION RENAL EXCRETION AND SURVIVAL

In Experiment I three groups of flounders (intact control, sham-operated and hypophysectomised fish) were prepared from fish caught in May, 1969 and maintained in sea water for two weeks. Blood samples were taken three weeks after surgery. At the same time urine samples were collected directly from the bladder. One week later the flounders were transferred from sea water into one-third sea water for an intermediate 12 hr period, thence into freshwater. A few fish began to show signs of stress (e.g. erratic swimming, skin lesions and impaired pigmentation) on the second day in freshwater, therefore blood and urine samples were taken from all the fish 48 hr after transfer. Several flounders from this stock were spent and in poor condition. Such fish may have been rather susceptible to the rigours of the experiment, particularly the effects of surgery, sampling and the fairly abrupt reversal of the osmotic gradient imposed by SW→FW transfer.

The data upon the blood haematocrit, and the osmolality and sodium content of the blood and urine of fish in Experiment I are given in Tables 2-4. The statistical treatment corresponded to that described below for Experiment II.

Stock flounders caught during October-November, 1969 were used in Experiment II which formed the major part of the investigation in Stirling. The fish were in good condition and survived up to six months in sea water. Groups of control, sham-operated and hypophysectomised fish were fitted with bladder catheters. Following hypophysectomy a minimum of two weeks in sea water elapsed before the withdrawal of blood samples. A further two weeks later the flounders were transferred into freshwater (with an intermediate 12 hr in one-third sea water). Signs of stress became evident in a few hypophysectomised fish after ten days in freshwater. Blood samples were taken from all fish on the eleventh day.

In Experiment II the rate of urine flow was determined. In sea water the urine samples were collected over *circa* 60 hours, one or two days after blood sampling. The urine production was meagre and the catheters frequently became blocked with deposits of secreted solids. In order to obtain samples adequate for ion analyses it was then necessary to repeat the urine collections over two or three days. Blood was collected before urine to avoid the possibility that stress induced by urine sampling might have influenced the composition of the blood.

In freshwater the urine flow was copious and samples were collected over short periods (<24 hr) two days before blood sampling.

Data from the blood and urine analyses and urine flow measurements in Experiment II are given in Tables 5-10. The data for each parameter (e.g. plasma sodium) were analysed statistically with techniques described by Snedecor (1956) and Bailey (1959). Initially a two-way analysis of variance (mixed, unequal sized sets) was used to assess the effects exerted by surgical treatment, environmental transfer, and interactions of these two components. It was found that SW→FW transfer in Experiment II produced significant reductions ($P < 0.01$) in the osmolality and ionic composition (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-) of the blood and urine of flounders in each group. The effects of hypophysectomy in each environment were investigated further with a one-way analysis of variance. Following the detection of a significant effect ($P < 0.05$) for a particular parameter the source of the difference was isolated by Student's 't' test. In no instance was a significant difference between intact control and sham-operated fish detected, demonstrating that the surgical techniques involved in hypophysectomy did not influence osmoregulation. The significance levels quoted in Tables 2-10 were derived from a valid comparison of sham-operated and hypophysectomised fish.

Heterogeneous variances between groups were detected occasionally whereupon the statistical tests were modified to include a weighted mean in analysis of variance

(Snedecor, 1956) and a subsequent 'd' test (Bailey, 1959). The use of these devices was found not to alter the final significance level. It is concluded that the analysis was valid and the anomaly was attributed to sampling phenomena.

In Experiment III hypophysectomised flounders in freshwater were treated with ovine prolactin and the effects of the hormone upon the osmolality and sodium content of the blood were determined.

Haematocrit - Experiments I & II

The data upon blood haematocrit from Experiments I & II are given in Table 2. The haematocrits of the two fish stocks used in these studies were similar, ranging from 17-20% in sea water and slightly higher, 18-23% in freshwater. These values were low when compared with the mean haematocrits of 30% and 32% in marine and freshwater teleosts respectively quoted by Thorson (1961). Possible dietary deficiencies in fish held in stock and starvation during the course of experiments may have provoked an anaemia. An additional possibility is that the low environmental temperature (10°C) led to a reduction in haematocrit, an effect observed in other teleosts (Anthony, 1961; Hevesy et al, 1964). The tendency for haematocrit to increase slightly upon transfer from sea water to freshwater agreed with Thorson's finding. In sea water (Experiment II) hypophysectomy provoked a slight aggravation ($P < 0.01$) of the anaemia.

Table 2

Experiments I and II

Haematocrit (% erythrocytes in whole blood by volume)

	Controls	Shams	Hypecs
SW	19 ± 1.2 (10)	19 ± 1.2 (7)	18 ± 0.9 (11)
FW (48 hr)	21 ± 1.3 (10)	20 ± 1.1 (7)	18 ± 1.4 (11)
SW	20 ± 1.0 (13)	20 ± 0.6 (12)	17 ± 0.8** (11)
FW (11 days)	21 ± 1.0 (13)	21 ± 1.2 (12)	23 ± 2.2 (11)

Means ± S.E.

Number of fish in parentheses

** $P < 0.01$

Experiment I

Blood and urine composition

The data upon the composition of blood and urine are summarized in Tables 3 and 4. Hypophysectomy did not affect the serum osmolality or plasma sodium content of flounders in sea water. Slight variations in the means for control, sham-operated and hypophysectomised fish were not significant. In each group plasma sodium contributed 49-50% of the total osmolality.

After 48 hr in freshwater (external sodium < 0.2 meq/l) significant reductions ($P < 0.05$) in the osmolality and sodium content of the blood were apparent in the three groups of flounders. The respective reductions in serum osmolality and plasma sodium were: controls 11.3%, 9.6%; shams 6.5%, 4.3%; and hypecs 12.3%, 12.0%. The contribution of sodium ions towards total osmolality remained at 50% and their decline (in meq/l) accounted for one half of the change in osmolality. Therefore a concomitant reduction in a plasma anion (that is, chloride) is assumed to have occurred. The reductions in osmolality and sodium content were most extensive in the hypophysectomised flounders. In the latter group these parameters were significantly lower ($P < 0.05$) than in the sham-operated fish (though their differences from the control group were of a lesser extent).

Urine osmolality showed a marked individual variation in each environment. In sea water the urine was approximately isosmotic or hypoosmotic to the serum. The means

Table 3

Experiment I

The effects of hypophysectomy upon blood and urine composition of the flounder adapted to sea water, transferred to 1/3 SW (12 hr), thence to FW and sampled after 48 hr

	Sea Water			Freshwater		
	Serum osmolality mOsm/kg	Plasma Na^+ meq/1	Urine osmolality mOsm/kg	Serum osmolality mOsm/kg	Plasma Na^+ meq/1	Urine osmolality mOsm/kg
Controls (n = 10)	340.2 ± 4.34	167.2 ± 1.20	284.7 ± 11.69	302.2 ± 4.60	151.1 ± 2.46	117.3 ± 22.96
Shams (n = 7)	332.6 ± 1.23	165.4 ± 1.86	299.1 ± 18.30	311.1 ± 4.25	158.1 ± 2.30	92.4 ± 11.93
Hypecs (n = 11)	331.8 ± 2.77	165.9 ± 1.64	291.2 ± 10.15	291.0 ± 5.15*	145.0 ± 2.78**	138.3 ± 15.96

Means ± S.E. * $P < 0.05$, ** $P < 0.01$

Table 4

Experiment I

Urine Sodium Content

	U_{Na} in sea water meq/l	U_{Na} in freshwater meq/l
Controls	72.1 ± 21.5	45.8 ± 10.6
	(15.3 - 137.2)	(11.7 - 131.6)
	(n = 7)	(n = 10)
Shams	60.0 ± 21.0	38.1 ± 4.7
	(5.8 - 102.1)	(14.9 - 50.6)
	(n = 4)	(n = 7)
Hypecs	96.2 ± 22.4	56.4 ± 8.8
	(10.0 - 153.0)	(22.9 - 124.4)
	(n = 7)	(n = 10)

Means ± S.E. with range

n = number of fish

for the three groups of fish were similar. The urine osmolality was reduced by 60% after transfer to freshwater, becoming markedly hypoosmotic to the serum.

Urine sodium determinations (Table 4) were incomplete since the sampling method gave a poor yield. In sea water the urine sodium content was highly variable in each group of flounders. The decline in urine sodium in freshwater was circa 40%, rather less than that for osmolality indicating alterations in the other ionic components of urine. Hypophysectomy did not influence the urine sodium content of flounders in either environment.

Experiment II

The data in Tables 5-10 summarize the various parameters measured for flounders in sea water and freshwater. The osmolality and Na^+, K^+ and Cl^- content of blood were of major interest and these data are complete, including results for each fish in the two environments. Factors limiting the completion of the remaining data included the large quantities of plasma and urine required for divalent ion analyses and the low yield of urine from SW - adapted fish.

Blood composition

The means for serum osmolality in SW - adapted control and sham-operated flounders (Table 6) were almost identical but that for hypophysectomised fish was reduced significantly ($P < 0.05$). The plasma sodium content of the three groups was similar, but the reduced plasma chloride of

Table 5

Experiment II

The effects of hypophysectomy upon blood composition of the flounder in sea water

	Serum osmolality mOsm/kg	Plasma electrolytes meq/l				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Controls	335.0 ± 2.25 (13)	165.6 ± 2.78 (13)	3.58 ± 0.10 (13)	4.20 ± 0.14 (12)	1.22 ± 0.04 (12)	145.6 ± 2.65 (13)
Shams	336.3 ± 2.87 (12)	163.5 ± 2.17 (12)	3.54 ± 0.09 (12)	4.55 ± 0.17 (12)	1.34 ± 0.05 (12)	142.8 ± 1.05 (12)
Hypecs	327.7 ± 1.48* (11)	162.6 ± 2.27 (11)	3.34 ± 0.09 (11)	4.26 ± 0.13 (11)	1.47 ± 0.06 (10)	135.3 ± 1.32*** (11)

Means ± S.E. Number of fish in parentheses * P < 0.05, *** P < 0.001

hypophysectomised fish (7.5 meq/l lower than shams, $P < 0.001$) accounted for the corresponding difference in serum osmolality (8.6 mOsm/kg). Pituitary ablation did not affect the other plasma electrolytes (K^+, Ca^{2+}, Mg^{2+}) determined in SW - adapted fish. Assuming dissociation was complete the electrolytes determined (as mM/l) could account for 93-95% of the serum osmolality.

After eleven days in freshwater the osmolality and electrolyte content of the blood were reduced in the three groups of fish (Table 6). In control and sham-operated flounders the serum osmolality declined by 59 mOsm/kg and 54 mOsm/kg respectively, or circa 17%. These reductions were mediated by decreases in plasma sodium and chloride (controls Na^+ - 31.8 meq/l, Cl^- - 31.4 meq/l; shams Na^+ - 29.0 meq/l, Cl^- - 30.0 meq/l), approximately 20% for each ion. In freshwater those plasma electrolytes determined contributed 92-93% of the total osmolality.

In hypophysectomised flounders transferred to freshwater the reductions in serum osmolality and plasma sodium and chloride content were greater ($P < 0.01$) than in the two groups with the hypophysis intact. The reductions of 50.7 meq/l and 42.4 meq/l in plasma sodium and chloride respectively (or 31-32% for each ion) were responsible for the 90 mOsm/kg (or 28%) decline in osmolality.

The plasma potassium content of SW - adapted flounders was not affected by hypophysectomy; in freshwater the declines in the two groups of intact fish were variable.

Table 6

Experiment II

The effects of hypophysectomy upon blood composition of the flounder eleven days after transfer to freshwater

	Serum osmolality mOsm/kg	Plasma electrolytes meq/l				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Controls	275.9 ± 4.54 (13)	133.8 ± 1.25 (13)	3.25 ± 0.09 (13)	3.93 ± 0.10 (13)	0.72 ± 0.06 (12)	114.2 ± 2.71 (13)
Shams	272.6 ± 4.56 (12)	134.5 ± 1.75 (12)	2.93 ± 0.10 (12)	4.04 ± 0.10 (11)	0.84 ± 0.11 (11)	112.8 ± 2.90 (12)
Hypecs	237.5 ± 10.00** (11)	111.9 ± 4.75*** (11)	3.55 ± 0.05 (10)	3.25 ± 0.20 (10)	0.46 ± 0.10* (10)	92.9 ± 5.75** (10)

Means ± S.E. Number of fish in parentheses *P < 0.05, ** P < 0.01, *** P < 0.001

The apparent increase in the plasma potassium of hypophysectomised fish in freshwater was due to a single abnormally high value, 7.79 meq/l. Exclusion of this result, which arose from a haemolysed blood sample, gave a new mean, 3.08 ± 0.18 meq/l (n = 9). An analysis of variance upon the revised data revealed that SW → FW transfer reduced significantly ($P < 0.001$) the plasma potassium of the three groups of fish with no treatment (hypophysectomy) or interaction effects.

The plasma Na/K ratios of the three groups in sea water were similar (controls 46.8 ± 1.83, n = 13; shams 46.5 ± 1.39, n = 12; hypecs 49.0 ± 1.50, n = 11), but in freshwater a possible trend towards a reduction (controls 41.6 ± 1.31, n = 13; shams 46.6 ± 1.91, n = 12) was obscured by a high individual variation, though the Na/K ratio of hypophysectomised fish in this medium (39.0 ± 2.27, n = 9) was significantly lower ($P < 0.02$) than in sham-operated fish.

In SW - adapted flounders plasma calcium and magnesium were not affected by hypophysectomy. Transfer to freshwater was accompanied by significant reductions ($P < 0.001$) in the plasma content of both divalent ions in the three groups of fish. For calcium the decline was most marked in hypophysectomised fish (-17.4% as against - 6.4% in controls and -11.2% in shams) but the difference was not significant due to a marked individual variation. For magnesium the reductions were circa 40% in control and sham-operated fish. In hypophysectomised flounders the reduction in freshwater was 69%, plasma magnesium being lower ($P < 0.05$) than in the two control groups.

Renal Excretion

Urine flow The urine flow of SW-adapted flounders was low; individual variation was high but the means for the three groups were similar (Tables 7 & 8). Urine flow was not affected by hypophysectomy. The mean values (controls, 0.016 ml/hr/100 gm) were considerably less than those determined by other workers (0.054 ml/hr/100 gm, Henschel, 1936; 0.060 ml/hr/100 gm, Lahlou, 1967; 0.047 ml/hr/100 gm, Motais, 1967). The French authors collected urine over long periods (maximum 15 days, Lahlou) from fish held in individual aquaria at higher environmental temperature (16-18°C) than in the present investigation (10°C).

In freshwater the urine flow was seven to nine-fold higher ($P < 0.001$) than in sea water, and the group means were similar, hypophysectomy being without effect. The rates of flow (controls, 0.117 ml/hr/100 gm) were again rather lower than those reported previously (0.178 ml/hr/100 gm, Lahlou, 1967; 0.287 ml/hr/100 gm, Motais, 1967).

Urine composition In SW-adapted control and sham-operated flounders the means for urine osmolality (Table 7) are approximately equal to those for serum. However the variability of individual results was high and in nine intact flounders the urine was hyper-osmotic (+2 - +24 mOsm/kg) to the serum. The validity of this comparison may be queried since the blood and urine were not sampled simultaneously. However an ideal

Table 7

Experiment II

The effects of hypophysectomy upon urine flow and composition in the flounder in sea water

	Urine osmolality mOsm/kg	Urine electrolytes meq/l					Urine flow ml/hr/100 gm
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	
Controls	328.1 ± 11.99 (11)	111.7 ± 10.45 (12)	3.19 ± 0.25 (12)	10.70 ± 1.25 (11)	11.49 ± 2.10 (12)	132.9 ± 9.07 (12)	0.016 ± 0.0027 (10)
Shams	326.6 ± 5.82 (11)	85.2 ± 9.67 (11)	2.63 ± 0.29 (11)	15.55 ± 2.47 (10)	11.71 ± 1.75 (10)	131.8 ±12.68 (9)	0.015 ± 0.0025 (8)
Hypecs	264.2 ± 17.4** (11)	90.1 ± 13.84 (10)	2.88 ± 0.21 (9)	6.33 ± 0.56** (9)	7.51 ± 0.78 (9)	58.8 ±11.09** (8)	0.012 ± 0.0018 (8)

Means ± S.E. Number of fish in parentheses ** P <0.01

comparison to avoid the time delay after urine formation would entail not only ureter catheterization, which is impractical in fish, but also would negate the sodium reabsorptive function of the flounder bladder (Lahlou, 1967). It may be assumed that the serum osmolality remained fairly stable and hence that hyperosmotic urine were indeed secreted. This phenomenon is rare among teleosts and has not been reported previously in Platichthys flesus. It is suggested that stress effects evoked by the sampling technique were responsible. In Experiment I urine samples were collected directly from the bladder without prior handling and slightly hyperosmotic urines (+1 → +4 mOsm/kg) were recorded in only three of seventeen intact flounders held in sea water. The phenomenon was observed following abrupt FW → SW transfer in Fundulus kansae (Stanley and Fleming, 1964; Fleming and Stanley, 1965) and bladder catheterization in Paralichthys lethostigma (Hickman, 1968b).

Both the means and ranges for the urine sodium of intact flounders adapted to sea water during Experiment I were lower than in Experiment II. Furthermore the data upon urine osmolality and sodium content in Experiment I correlated well with Lahlou's results (275 mOsm/kg, 60 meq/l) from long-term measurements involving little stress. The higher urine sodiums of Experiment II relate to the increased frequency of hyperosmotic urine secretion. In marine teleosts the predominant cation present in the urine is magnesium arising from the tubular secretion of ions absorbed

from ingested sea water. Sodium ions in the plasma ultrafiltrate are reabsorbed strongly in the renal tubule (Hickman and Trump, 1969). In the present investigation possible factors leading to the displacement of magnesium (controls, 11.5 meq/1) by sodium (111.7 meq/1) might have included (a) an increase in urine sodium to replace magnesium at low rates of urine flow (< 0.02 ml/hr/100 gm in Paralichthys lethostigma, Hickman, 1968b), (b) a marked increased urine sodium following handling (Hickman, 1968b), or (c) a partial abolition of sodium reabsorption in the bladder (Lahlou, 1967). Certainly the presence of the catheter would have prevented the accummulation of bladder urine thereby reducing active sodium retention by this organ.

Glomerular filtration rates were not determined in this investigation but Lahlou (1967) found that approximately 75% of the water in the plasma ultrafiltrate was reabsorbed in the tubule. Without the GFR the relative clearance and reabsorption of ions could not be calculated but the high U/P ratios (urine conc./plasma conc.) for calcium (controls 2.6, shams 3.4) and magnesium (9.4 and 8.8 respectively) indicated that divalent ions arose from active tubular secretion. As in other teleosts chloride was the major anion present in the urine. The crystalline precipitates in the urine of SW - adapted flounders were not analysed but would have consisted of salts with low solubility products, probably phosphates or bicarbonates of Ca^{2+}/Mg^{2+} ($CaHPO_4$ in Paralichthys lethostigma, Hickman, 1968b).

In hypophysectomised flounders adapted to sea water urine osmolalities were highly variable but the mean was circa 60 mOsm/kg lower than in the two intact groups. A hyperosmotic urine was recorded in only one fish (+24 mOsm/kg). The mean urine chloride was 54% lower ($P < 0.01$) than in sham-operated fish and this difference (72 meq/l) was adequate to account for the reduced osmolality. A corresponding reduction in a urine cation was not evident but that in chloride may be correlated with the low plasma chloride of hypophysectomised fish (Table 5). These data upon urine osmolality and chloride content should be contrasted with those of Experiment I where hypophysectomy did not affect osmolality (chloride not being determined). The mean urine osmolality of the ablated fish in Experiment II was lower than in Experiment I, but the differences between intact groups were more extensive. The data are difficult to interpret since in all groups the variability was high. However it is suggested that the high urine osmolality of intact flounders in Experiment II, possibly arising from stress effects, could have exaggerated the effect of hypophysectomy upon urine composition. The virtual absence of hyperosmotic urines in ablated flounders appeared to be mediated by a reduction in chloride, but the involvement of natriuretic effects in this phenomenon was evident from a comparison of the intact groups in Experiments I & II.

In SW - adapted flounders the sodium, potassium and magnesium content of the urine was not affected by hypophysectomy, but urine calcium was reduced ($P < 0.01$).

Presumably a decreased tubular secretion of calcium mediated this effect but plasma calcium was not affected by the ablation.

After *circa* nine days in freshwater (Table 8) the increase in urine flow in the three groups of flounders was accompanied by a marked reduction (65 - 75%) in urine osmolality. The latter was mediated mainly by decreases in urine sodium (60 - 70%) and chloride (60 - 80%). Substantial reductions in the divalent ions, calcium and magnesium, were to be expected since their main source is ingested sea water. Urine magnesium should attain very low levels upon the completion of renal adaptation to freshwater. Lahlou (1967) found that the main adjustments in urine flow and composition occurred within 24 hr of SW→FW transfer, but a steady decline in urine sodium and chloride remained evident after three days. The sodium, magnesium and chloride content and the flow of urine were not affected by hypophysectomy, but urine potassium and calcium were increased by the ablation.

Ion efflux rates The renal efflux rates for the ions Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- given in Tables 9 & 10 were calculated as μeq/hr/100 gm from data upon the urine flow and composition of individual flounders. An indication of total electrolyte losses via the urine was derived from the osmolar efflux in μOsm/hr/100 gm. In SW-adapted flounders the low rates of electrolyte excretion were a function of the meagre urine flow.

Table 8

Experiment II

The effects of hypophysectomy upon urine flow and composition in the flounder in freshwater

	Urine osmolality mOsm/kg	Urine electrolytes meq/l					Urine flow ml/hr/100 gm
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	
Controls	86.0 ± 12.4 (13)	36.7 ± 6.86 (13)	1.37 ± 0.15 (13)	3.97 ± 0.67 (13)	2.21 ± 0.41 (13)	24.2 ± 3.76 (13)	0.117 ± 0.0099 (13)
Shams	107.0 ± 18.48 (12)	34.4 ± 7.97 (12)	1.15 ± 0.12 (12)	2.87 ± 0.35 (12)	2.91 ± 0.53 (12)	21.8 ± 7.37 (12)	0.124 ± 0.0137 (12)
Hypecs	93.0 ± 8.23 (11)	30.4 ± 3.33 (11)	2.43 ± 0.29** (10)	5.52 ± 0.73** (10)	4.02 ± 0.58 (10)	23.1 ± 5.64 (11)	0.117 ± 0.0190 (10)

Means ± S.E. Number of fish in parentheses ** P < 0.001

Table 9

Experiment II

Renal depletion of electrolytes in sea water

	Osmolality µOsm/hr/100gm	Electrolytes µeq/hr/100 gm				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Controls	5.4 ± 1.2 (8)	2.3 ± 0.8 (9)	0.048 ± 0.007 (9)	0.187 ± 0.043 (10)	0.214 ± 0.063 (10)	2.1 ± 0.4 (9)
Shams	4.7 ± 0.8 (8)	1.2 ± 0.2 (8)	0.034 ± 0.006 (8)	0.250 ± 0.067 (8)	0.193 ± 0.050 (8)	2.3 ± 0.6 (7)
Hypecs	3.3 ± 0.6 (9)	1.0 ± 0.3 (9)	0.037 ± 0.005 (9)	0.077 ± 0.011* (9)	0.096 ± 0.015 (9)	0.7 ± 0.2 (7)

Means ± S.E. Number of fish in parentheses * P <0.05

Table 10

Experiment II

Renal depletion of electrolytes in fresh water

	Osmolality µOsm/hr/100gm	Electrolytes µeq/hr/100 gm				
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Controls	9.3 ± 0.9 (13)	3.9 ± 0.5 (13)	0.152 ± 0.014 (13)	0.468 ± 0.097 (13)	0.295 ± 0.069 (12)	2.6 ± 0.4 (12)
Shams	12.8 ± 1.9 (12)	3.5 ± 0.6 (12)	0.136 ± 0.020 (12)	0.354 ± 0.056 (12)	0.374 ± 0.087 (12)	2.0 ± 0.5 (12)
Hypecs	10.2 ± 1.8 (10)	3.5 ± 0.7 (9)	0.304 ± 0.059** (10)	0.633 ± 0.169 (9)	0.392 ± 0.050 (9)	2.7 ± 0.7 (10)

Means ± S.E. Number of fish in parentheses ** P <0.01

The renal sodium efflux of intact fish (controls 2.3 μeq/hr/100 gm) was comparable to values obtained previously (2-4 μeq/hr/100 gm, Lahlou, 1967; Motais, 1967). The reduced calcium and chloride content of the urine of hypophysectomised fish was reflected in the low efflux rates for these ions but due to the variable urine flow only the calcium efflux was significantly lower ($P < 0.05$)) than in sham-hypophysectomised fish.

In freshwater the osmolal and ionic renal effluxes were slightly higher than in sea water. The sodium fluxes (3.5 - 3.9 μeq/hr/100 gm) of intact fish corresponded to other values obtained in freshwater (5-6 μeq/hr/100 gm, Lahlou, 1967; Motais, 1967). The extensive increase in urine flow (circa x 8) in the hypoosmotic environment but relatively minor rises in electrolyte depletion indicated a substantial, but not fully compensatory, increase in the tubular reabsorption of monovalent ions. The renal effluxes of sodium, magnesium and chloride were not affected by hypophysectomy but potassium efflux was doubled ($P < 0.001$). The calcium efflux, though highest in the ablated group, did not differ significantly from the rates in intact fish.

Freshwater Survival

After the collection of blood from flounders in freshwater the maximum survival period in this medium was determined. The results are given in Table 11. The first death of a hypophysectomised fish occurred on the eleventh day after SW→FW transfer. One fish from this group survived to the twenty-fourth day.

Table 11

Experiment II

Hypophysectomy and freshwater survival in the flounder

	Controls	Shams	Hypecs
FW Survival days	22 ± 1 (13)	24 ± 1 (12)	16 ± 1.5** (11)

Means ± S.E.

Number of fish in parentheses

** $P < 0.01$

Table 12

Experiment III

The effect of ovine prolactin upon blood composition of hypophysectomised flounders in freshwater

Prolactin dose: 100 mU/gm.wt. (circa 3 µg/gm.wt.)

	Serum osmolality mOsm/kg	Plasma Na^+ meq/l
Hypecs + 0.8% saline (n = 5)	288.2 ± 12.61	137.2 ± 4.57
Hypecs + prolactin (n = 6)	273.4 ± 7.33	130.3 ± 3.20
P	n.s.	n.s.

Indications of imminent death included a reduced branchial ventilation rate, skin lesions, impaired pigmentation and muscular turgidity. Amongst the two control groups the survival period ranged from thirteen to thirty-two days. The deaths of several intact fish were associated with a temporary (4-6 hours) 5°C rise in water temperature caused by a faulty refrigeration unit. A post-mortem examination of the brain confirmed that sham-operated and hypophysectomised fish had been designated correctly. Under the experimental regime described hypophysectomy impaired the freshwater survival of the flounder.

Experiment III

Ovine prolactin treatment

A group of hypophysectomised flounders were transferred from sea water into freshwater three weeks after surgery. Six fish received a course of three injections (100 mU or *circa* 3 µg/gm) of ovine prolactin (NIH-P-S9) dissolved in 0.8% saline. As a control five flounders were injected with saline alone. Blood samples were collected upon the seventh day in freshwater. The data upon serum osmolality and plasma sodium analyses are given in Table 12. There was a high individual variation within each group. Neither parameter was affected by treatment with ovine prolactin.

ISOTOPIC KINETIC STUDIES

This investigation was made in Villefranche-sur-Mer during February - April, 1970. Groups of sham-operated and hypophysectomised flounders were transferred from Mediterranean sea water (37‰, 520 meq Na/l) into one-third sea water and finally into freshwater (0.2 meq Na/l). Using the radioisotope ^{24}Na the sodium balance of intact and ablated flounders was compared in each environment. Tritiated water (HTO) was used in a parallel study upon the diffusional water permeability of flounders. The statistical significance of the effects of hypophysectomy upon these parameters was assessed with the Student's 't' test.

Effects of hypophysectomy on the dynamics of sodium and water balance

Sodium exchange in sea water

The data for sodium turnover (Table 13) are the means of repeat measurements (two or three) upon individual flounders. Single determinations of the sodium space and total ion efflux were made for each fish.

The sodium turnover, 45.1%/hr and efflux 2757 µeq/hr/100 gm of sham-operated flounders were in close agreement with earlier determinations, 49.6%/hr, 41%/hr and 2600 µeq/hr/100 gm (Motais, 1967; Evans, 1968 unpublished). The sodium space, 45.9 ml/100 gm (representing the volume of the

Table 13

Parameters concerning sodium metabolism measured in sea water

	Shams	Hypecs	
Sodium turnovers %/hr	45.1±3.01 (n = 10)	35.4±3.05 (n = 10)	P < 0.05
Sodium f_{out} µEq/hr/100 gm	2757±296 (n = 10)	2128±146 (n = 9)	n.s.
Sodium space ml/100gm	45.9±3.03 (n = 10)	40.1±2.44 (n = 9)	n.s.

Table 14

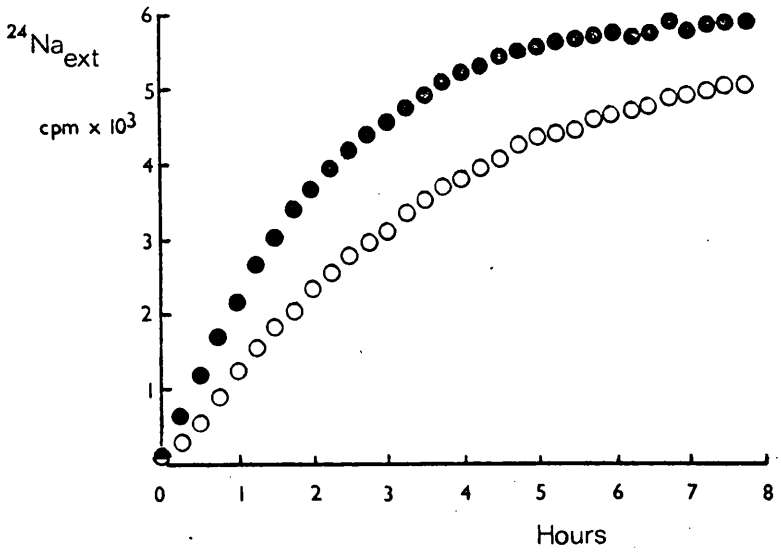
Sodium turnover in one-third sea water

	Shams	Hypecs	
λ_{Na} %/hr	3.3±0.79 (n = 8)	3.0±0.51 (n = 5)	n.s.

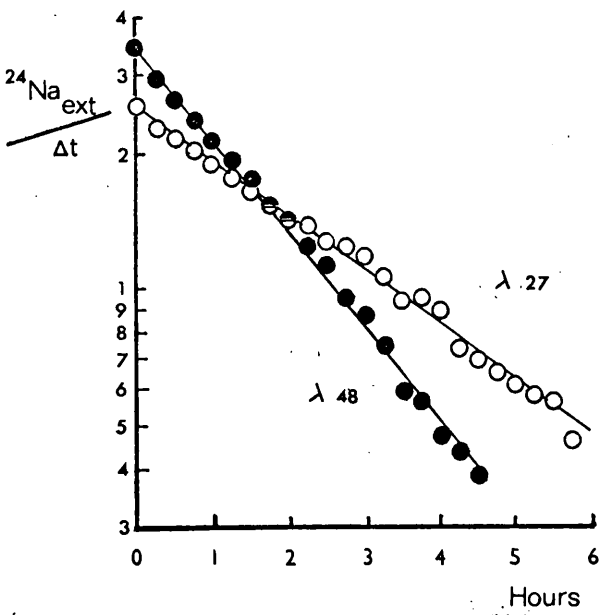
Figure 1.

(a) ^{24}Na evolution curves from a sham-operated (●), and hypophysectomised (o) flounder in sea water with (b) semi-logarithmetic plots to determine the rate constants λ_{Na}(%/hr) for sodium turnover.

(a)


$^{24}Na_{ext}$
cpm x 10^3
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
Hours

(b)


$^{24}Na_{ext}$
Δt
4
3
2
1
9
8
7
6
5
4
3
0
1
2
3
4
5
6
Hours
λ 27
λ 48

internal pool of exchangeable sodium) was higher than Motais' value, 33 ml/100 gm (determined by two methods at 17°C), but lower than Evans' 54.5 ml/100 gm for flounders adapted to 7°C for one week. Assuming an internal sodium concentration of 164 meq/l (Table 5) the exchangeable ion pool would equal 7.6 meq/100 gm. From this value knowing the sodium turnover, a theoretical efflux, 3500 μeq/hr/100 gm was calculated, which is high by comparison with the real value. Closer agreement is obtained with Motais' value for sodium space which gave a pool of 5.4 meq/100 gm and a theoretical efflux of 2460 μeq/hr/100 gm. The renal sodium efflux of SW-adapted fish was 2.3 μeq/hr/100 gm (Table 9) which was negligible before the total efflux and demonstrated that the main site for sodium extrusion was extrarenal, presumably at the branchial epithelium.

In Figure 1 the evolution curves for ^{24}Na and their graphical analyses are plotted for a sham-operated and a hypophysectomised flounder. These two fish were of similar weight, had received the same dose of radioactivity and had been immersed in identical volumes of sea water. Therefore their ^{24}Na curves are comparable. The evolution of radiosodium and its tendency to attain equilibrium were faster in the intact flounder, indicating that hypophysectomy reduced sodium exchange. The data in Table 13 show that hypophysectomy depressed the rate of sodium turnover by one-fifth below that for intact fish ($P < 0.05$). The reduction in sodium efflux was similar in extent (-23%) but this difference was not significant since the fluxes, determined only once in each fish, exhibited a high

individual variation.

A reliable differentiation between the effects of hypophysectomy upon the various components of sodium efflux (that is exchange diffusion, net excretion and passive fluxes) was not possible from this experiment. However it was evident from the extent of the reduction in sodium turnover that the Na:Na exchange diffusion mechanism was depressed since this component contributes 85-90% of the total exchange (Motais et al, 1966; Motais, 1967). Evidence of a close linkage between the exchange diffusion and net excretion components (Maetz et al, 1969a,b; Maetz, 1969b) indicates that the efficiency of the sodium pump also would have been reduced by hypophysectomy.

Sodium exchange in one-third sea water

Sodium turnovers were determined for flounders adapted to one-third sea water (12‰, 120 meq Na/1) for one week. The sodium turnover of sham-operated fish (Table 14) was 3.3%/hr, considerably less than the rate in sea water. Assuming a plasma sodium of 160 meq/1 and a sodium space of 33 ml/100 gm the exchangeable sodium pool would equal 5.4 meq/100 gm and the efflux, 180 μeq/hr/100 gm. These values are comparable with the slightly lower turnover, 3.1%/hr and efflux, 160 μeq/hr/100 gm determined for flounders in one-fourth sea water by Motais (1967).

The mean sodium turnover of hypophysectomised flounders was 3.0%/hr, giving a theoretical efflux of 160 μeq/hr/100 gm. Individual results in each group were variable and the slight difference between the means for the sodium exchange rate in intact and ablated fish was not significant.

Rapid transfer Experiments

Regulation of sodium efflux upon SW → FW → FW + K^+ → SW transfer In Figure 2 the ^{24}Na evolution traces represent the alterations in sodium efflux produced by the abrupt, short-term transfer of two SW-adapted flounders (sham-operated and hypophysectomised) into freshwater, freshwater enriched with potassium (10 mM K^+) and finally back into sea water. The means for the sodium efflux of sham-operated and hypophysectomised flounders transferred in this manner are given in Table 15.

An instantaneous reduction of 93% in the sodium efflux occurred upon the SW→FW transfer of sham-operated fish. The remaining efflux in freshwater probably represented a predominantly passive flux of sodium ions. The major part of the reduction upon transfer into freshwater (<0.2 meq Na/l) was due to the abolition of the Na:Na exchange diffusion component (Motais et al, 1966). In earlier measurements an 85-90% reduction in sodium efflux was recorded upon the SW→FW transfer of intact flounders (Motais et al, 1966; Motais, 1967). The 84% reduction observed in the hypophysectomised flounders was significantly less efficient ($P < 0.001$) than in sham-operated fish.

Transfer into FW + K^+ enhanced the sodium efflux of both intact and ablated flounders. In freshwater the Na:K exchange pump, which is the normal mechanism for active sodium extrusion in sea water (Maetz, 1969b), would have been abolished due to the absence of external potassium ions. Transfer into a potassium-rich (10 mM K^+, as in SW) medium permitted the reappearance of the Na:K pump. Assuming

Figure 2.

Alterations in the sodium efflux of a sham-operated (●), and hypophysectomised (o) flounder upon SW→FW→FW + 10mM K^+→SW transfer with intermediate rinse periods (see Table 15).

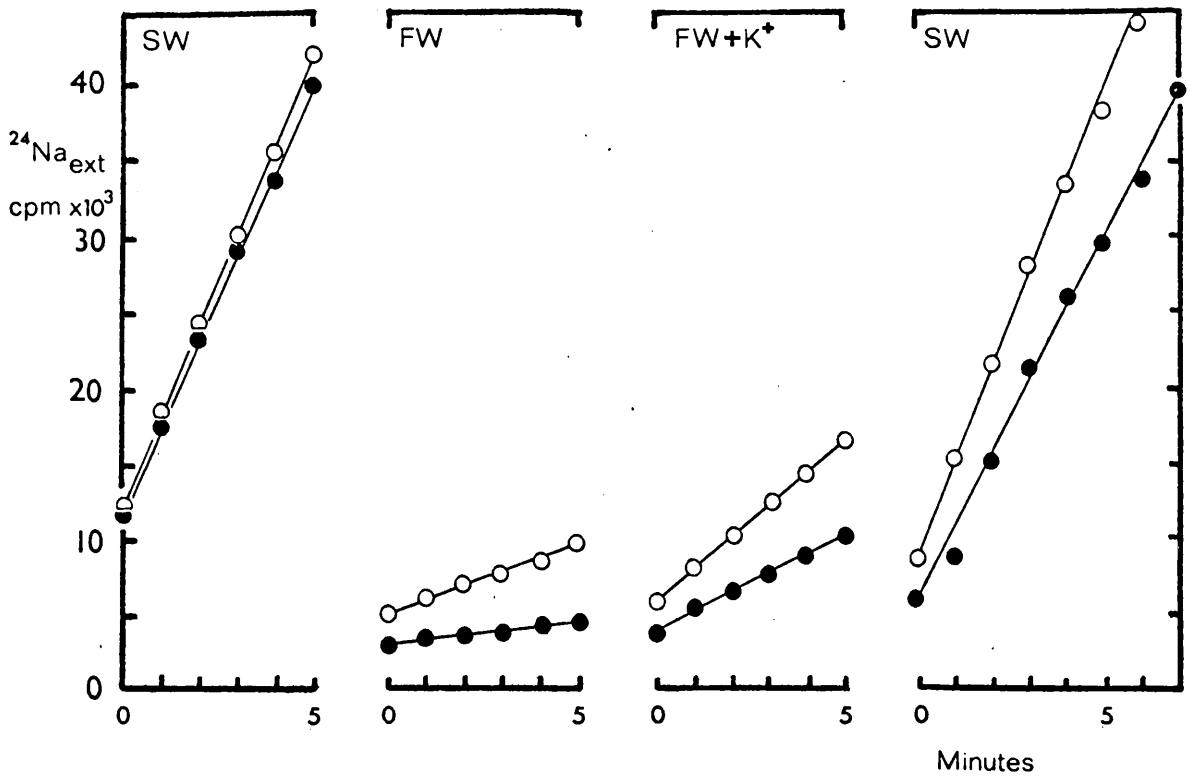

SW
FW
FW+K+
SW
40
30
20
10
0
24Naext
cpm x10^3
0
5
0
5
0
5
0
5
Minutes

Table 15

Instantaneous regulation of sodium efflux
and the Na:K exchange pump in SW-adapted fish
transferred SW→FW→FW+K^+→SW

	f_{out} in SW		f_{out} in FW		f_{out} in FW+10mM K^+	
	%	µeq/hr/100gm	%	µeq/hr/100gm	%	µeq/hr/100gm
Shams	100	2757	7.4±1.10 (n = 5)	204	29.1±2.97 (n = 5)	802
Hypecs	100	2128	16.1±0.86*** (n = 5)	342	40.2±1.90* (n = 5)	854

f_{out} expressed as a % of the control value in SW and calculated as absolute efflux (µeq/hr/100gm) relative to previous determinations (Table 13).

* P <0.02 *** P <0.001

that the K^+-induced sodium efflux was superimposed upon the flux already observed in freshwater it was evident that the enhancement in hypophysectomised flounders (+ 512 µeq/hr/100 gm) was less than that in the intact fish (+ 598 µeq/hr/100 gm). This difference was not significant but it was probable that the activity of the Na:K exchange pump was impaired by hypophysectomy.

In this experiment short-term transfers were necessary in order to avoid the secondary regulation of sodium efflux that occurs with a more prolonged (0.5 hr) adaptation to freshwater. It was apparent that this delayed regulation did not occur since the slopes of the ^{24}Na evolution curves were similar in the initial and final periods in sea water (as in Figure 2).

Thus the exchange diffusion component of sodium efflux was depressed by hypophysectomy. This effect probably extended to the active sodium pump operating by Na:K exchange. The impaired Na:Na exchange of hypophysectomised flounders reduced the efficiency of the instantaneous reduction in sodium efflux upon SW→FW transfer and was associated with a high passive efflux of sodium ions in freshwater.

Instantaneous and delayed regulation of sodium efflux upon SW →FW →SW transfer. The ^{24}Na evolution curves for an intact flounder transferred from SW →FW →SW are shown in Figure 3. The trace for the initial period in sea water is complete, but that for the freshwater period lacks points from the intermediate rinses. Only the initial part of the trace for the final period in sea water is shown. In Table

Figure 3.

Instantaneous and delayed regulation of sodium efflux upon SW→FW→SW transfer of an intact flounder (see Table 16).

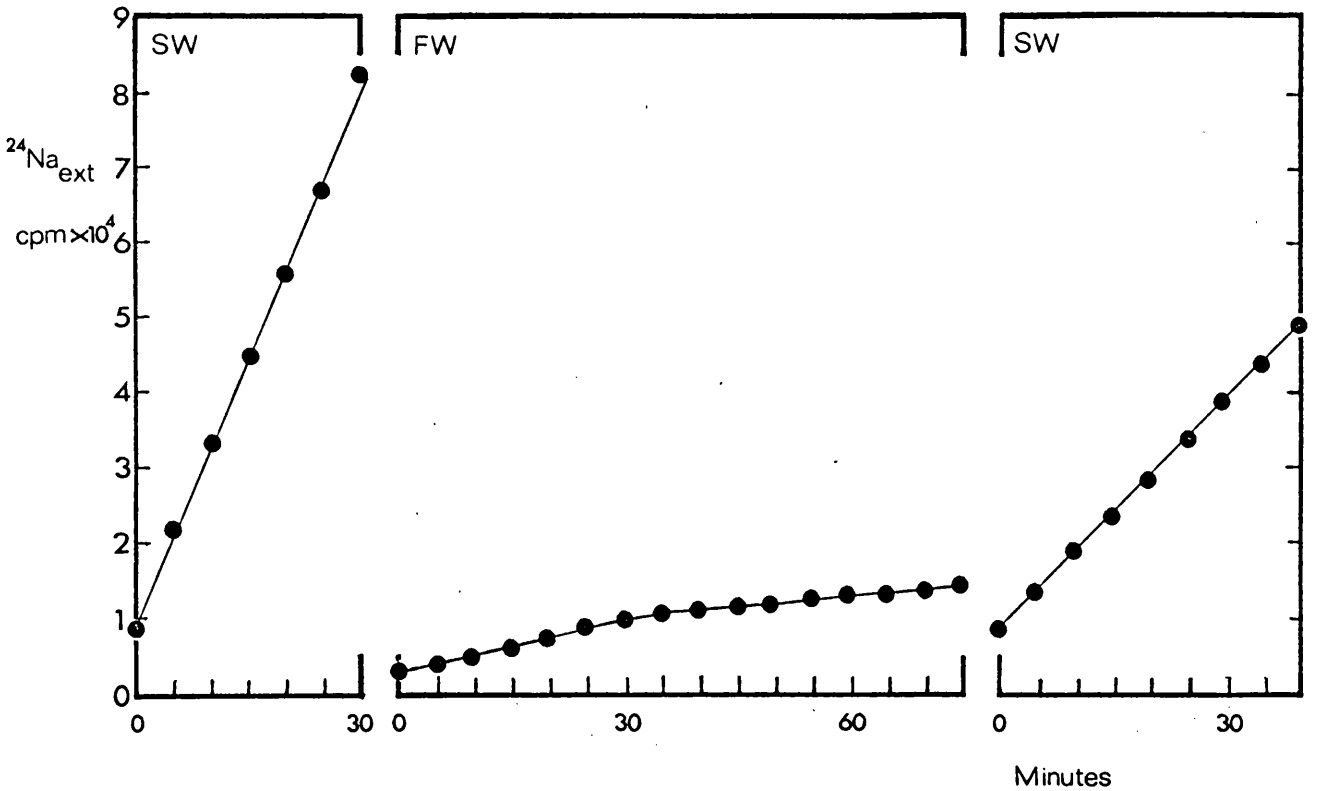

SW
FW
SW
$^{24}Na_{ext}$
cpm×10^4
0
1
2
3
4
5
6
7
8
9
0
30
0
30
60
0
30
Minutes

Table 16

Instantaneous reduction and secondary regulation of sodium efflux during SW→FW→SW transfer

	SW	FW		SW	
	long term adapted	0-0.5 hr	0.5-1.5 hr	0-1.25 hr	1.25-2.5 hr
Shams (n = 3)	2092 ± 299	192 ± 39	80 ± 3	1350 ± 76	1860 ± 235
Hypecs (n = 3)	1820 ± 215	168 ± 21	135 ± 28	1098 ± 64	1437 ± 222

f_{out} μeq/hr/100 gm

15 the sodium effluxes for sham-operated and hypophysectomised flounders are given as μeq/hr/100 gm.

In both groups of fish the instantaneous reduction in sodium efflux upon SW →FW transfer was circa 90%. As explained for the previous experiment the major part of this reduction was mediated by the abolition of Na:Na exchange diffusion fluxes. In the intact flounder (Figure 3) a reduction in sodium efflux in freshwater became apparent thirty minutes after transfer. The reduction corresponded to a secondary (or delayed) regulation of branchial sodium permeability (Motais et al, 1966; Motais, 1967). A similar trend was evident in both sham-operated and hypophysectomised flounders; sodium efflux underwent a substantial reduction in the second sub-period in freshwater (Table 16). This decrease in sodium permeability was not immediately reversible upon the return to sea water. In Figure 3 the final slope for external ^{24}Na is reduced in comparison with that for the initial period in sea water. In Table 16 the low sodium fluxes after FW→SW transfer (0-1.25 hr) were increased later (1.25-2.5 hr), as the rate of sodium exchange became progressively readapted to the marine environment. Motais (1967) found that the rate of readaptation was inversely related to both the length of the period spent in freshwater and the size of the fish.

In this experiment no significant differences were observed between sham-operated and hypophysectomised fish. However the number of fish used was small and

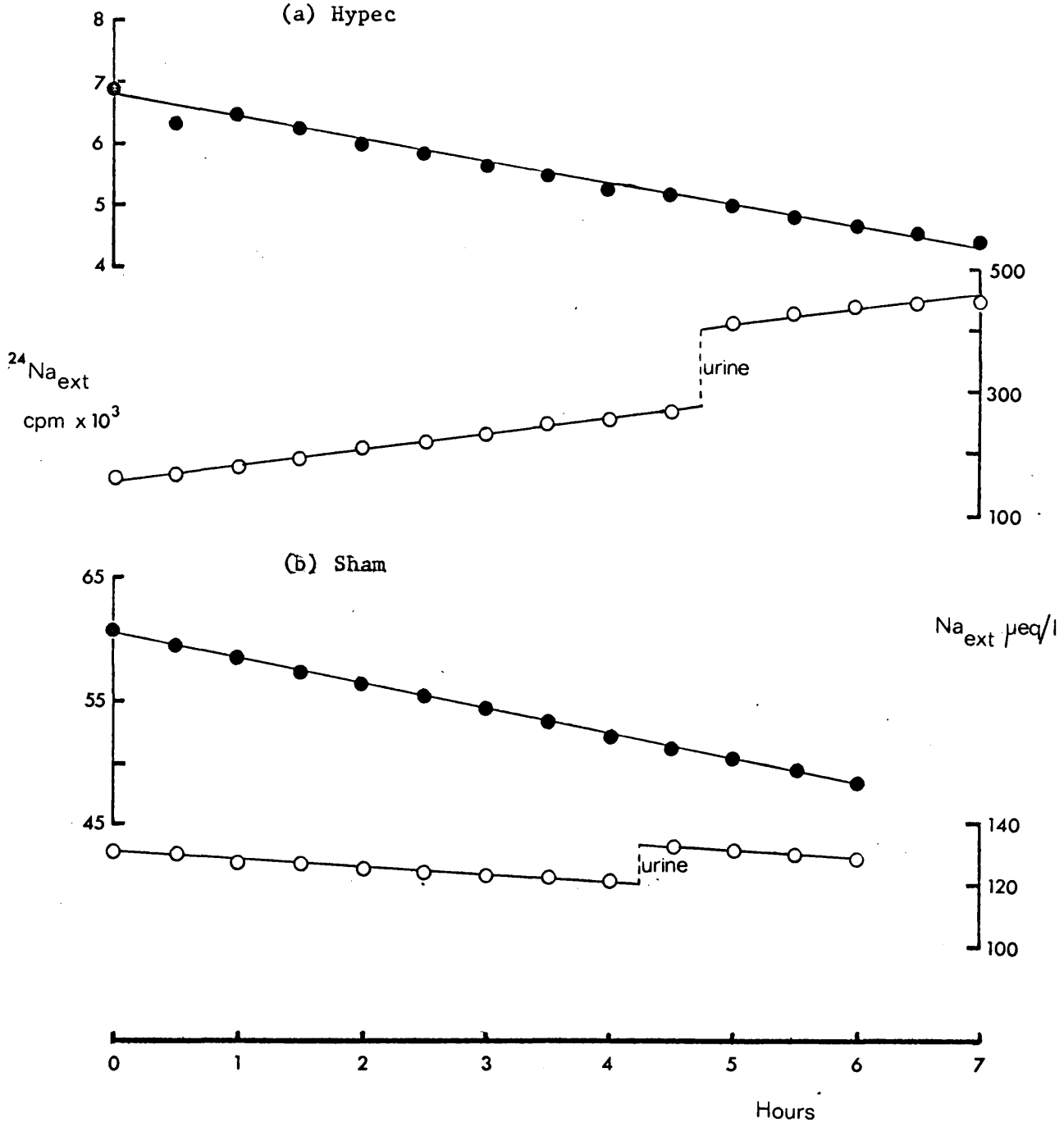
method of flux calculation necessarily indirect, hence the variability of the data.

Sodium balance in freshwater

Flux determinations The sodium fluxes of flounders were determined at various periods (1,5,9, 16 days) after 1/3 SW→FW transfer. Typical plots for the sodium influx and net flux are given in Figure 4. The efflux was calculated as the difference between these two components. The sham-operated flounder was in a state of positive sodium balance with external sodium in decline, whereas the hypophysectomised fish was in negative balance with a linear increase in external sodium. The abrupt increases in external ion concentration probably represented a sporadic release of urine. Their mean contribution to the sodium efflux from several experiments was 4.0 μeq/hr/100 gm, which agreed with the renal flux of 3.9 μeq/hr/100 gm determined previously by direct methods (Table 10). The slope of the plot, external sodium against time, was not altered following the release of urine and this extrarenal flux was presumably mediated by the gill membrane. In a few fish a slight but progressive reduction in the rate of the decline in external ^{24}Na occurred during the course of a measurement. The resultant shallow curve included a "back-flux" of radioactivity emerging from the fish. Estimations of its value in each case showed it to be negligible by comparison with the influx.

Figure 4.

Traces of external ^{24}Na (influx ●) and total external sodium (net flux ○) for (a) a hypophysectomised flounder and (b) a sham-operated fish, maintained in freshwater.



Fluxes µeq/hr/100gm	f_{in}	f_{net}	f_{out}	urine
Hypec 200gm, FW 5 days	+3.5	-7.6	-11.1	-5.2
Sham 98gm, FW 9 days	+3.4	+1.6	-1.8	-1.3

The main investigation of sodium balance in flounders in freshwater was made five days after 1/3 SW→FW transfer (Table 17, Figure 5). Both sham-operated and hypophysectomised flounders were in a state of negative sodium balance. In the ablated fish the net sodium efflux was higher ($P < 0.05$) than in their sham-operated controls. This difference was due to an augmentation ($P < 0.01$) of sodium efflux in the hypophysectomised fish; the rate was doubled by the ablation. The sodium influx of hypophysectomised flounders was slightly higher than in intact fish, but when influx was considered in relation to the external sodium concentration as $^{f}in/Na_{ext}$ the rates were similar in the two groups. The high sodium efflux of ablated fish led to a rise in external sodium in the closed circulation, which in turn facilitated the branchial absorption of sodium ions.

The data in Table 17 refer only to the branchial sodium fluxes. Upon inclusion of the renal fluxes the mean sodium effluxes were increased to 5.7 ± 1.50 μeq/hr/100 gm in sham-operated fish and 9.4 ± 0.53 μeq/hr/100 gm in hypophysectomised fish. The revised rates for the net efflux were -3.1 ± 1.84 μeq/hr/100 gm in intact fish and -4.3 ± 0.70 μeq/hr/100 gm in ablated fish. The variability was high since the release of urine was erratic and the experimental period short (<u>circa</u> 7 hr). Motais' (1967) values for the component fluxes of sodium in the FW-adapted flounder were slightly greater (f_{in} 14 μeq/hr/100 gm, f_{out} 22 μeq/hr/100 gm, f_{net} -8 μeq/hr/100 gm) but these

Table 17

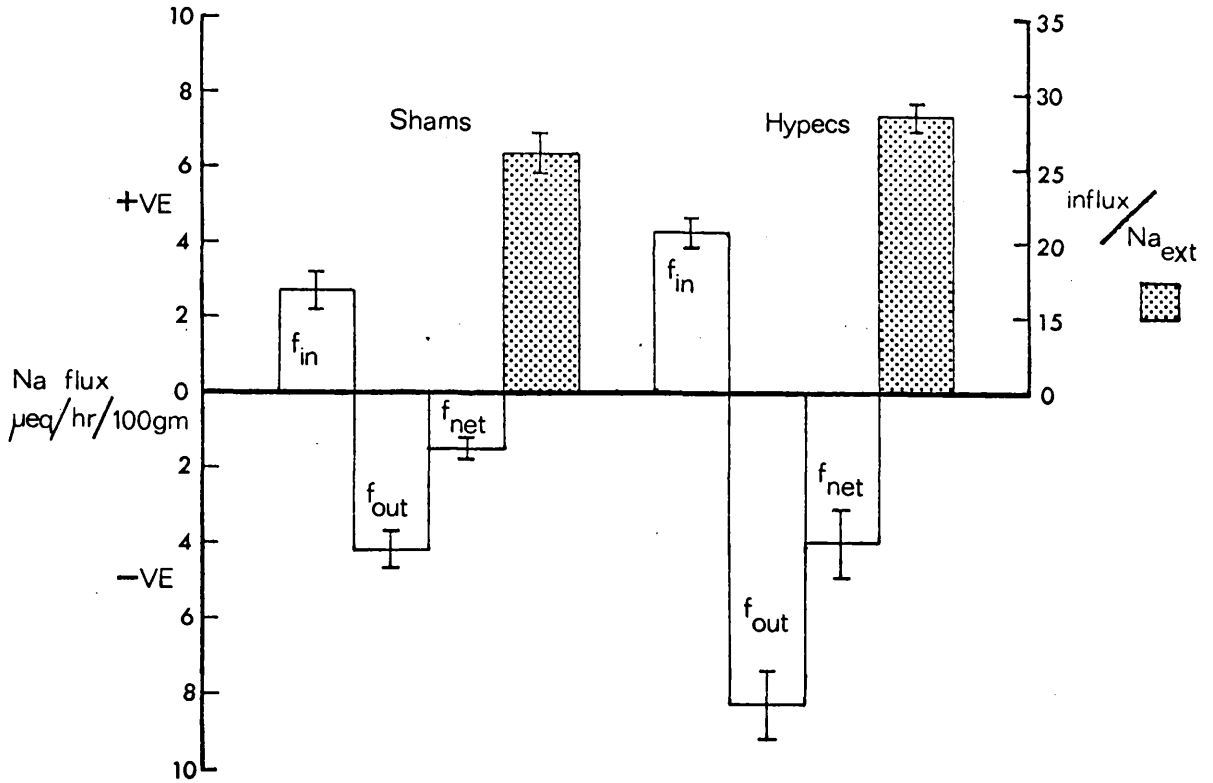
Sodium fluxes 5 days after transfer from one-third sea water to freshwater

	f_{in}	f_{net}	f_{out}	Na_{ext} mEq/l	f_{in}/Na_{ext}
Shams (n = 7)	2.7 ± 0.49	-1.5 ± 0.29	4.2 ± 0.50	0.172	16.0 ± 1.0
Hypecs (n = 7)	4.3 ± 0.37	-4.0 ± 0.85*	8.3 ± 0.91**	0.236	18.6 ± 1.7

* $P < 0.05$, ** $P < 0.01$ Fluxes μEq/hr/100 gm

Figure 5.

Sodium fluxes in sham-operated (n = 7) and hypophysectomised (n = 7) flounders after five days in freshwater (see Table 17).


Shams
Hypecs
+VE
−VE
Na flux
μeq/hr/100gm
influx / Na ext
f in
f out
f net
f in
f out
f net
10
8
6
4
2
0
2
4
6
8
10
35
30
25
20
15
0

measurements were made in a medium richer in sodium (0.46 meq/1). Using water of lower sodium content (0.1 - 0.2 meq/1) Motais obtained values (f_{in} 1-2 μeq/hr/100 gm, f_{out} 8-12 μeq/hr/100 gm) compatible with those of the present investigation.

Adaptation to freshwater The sodium fluxes of flounders adapted to freshwater for various periods are given in Table 18 and illustrated in Figure 6. Data from hypophysectomised fish injected with prolactin are included, but their intact controls received 0.8% saline alone. The sodium fluxes of sham-operated fish underwent a progressive modification until the mean net flux became positive (though not significantly above zero) after sixteen days in freshwater. This favourable sodium balance was mediated by an increased ion influx for the efflux was reduced only slightly. An enhanced efficiency in branchial sodium absorption was evident from the marked increase in the ratio $^{f}in/Na_{ext}$.

The rate of sodium depletion in hypophysectomised flounders was reduced with longer periods in freshwater, but the mean net flux remained negative. This negative sodium balance was due primarily to a persistent high efflux since the influx and the efficiency of branchial sodium absorption ($^{f}in/Na_{ext}$) increased as in the intact flounders.

In freshwater flounders the main effect of hypophysectomy was to increase sodium efflux. It is probable that the passive permeability of the branchial membrane

Table 18

Sodium fluxes at various periods after tranfer from one-third sea water to freshwater

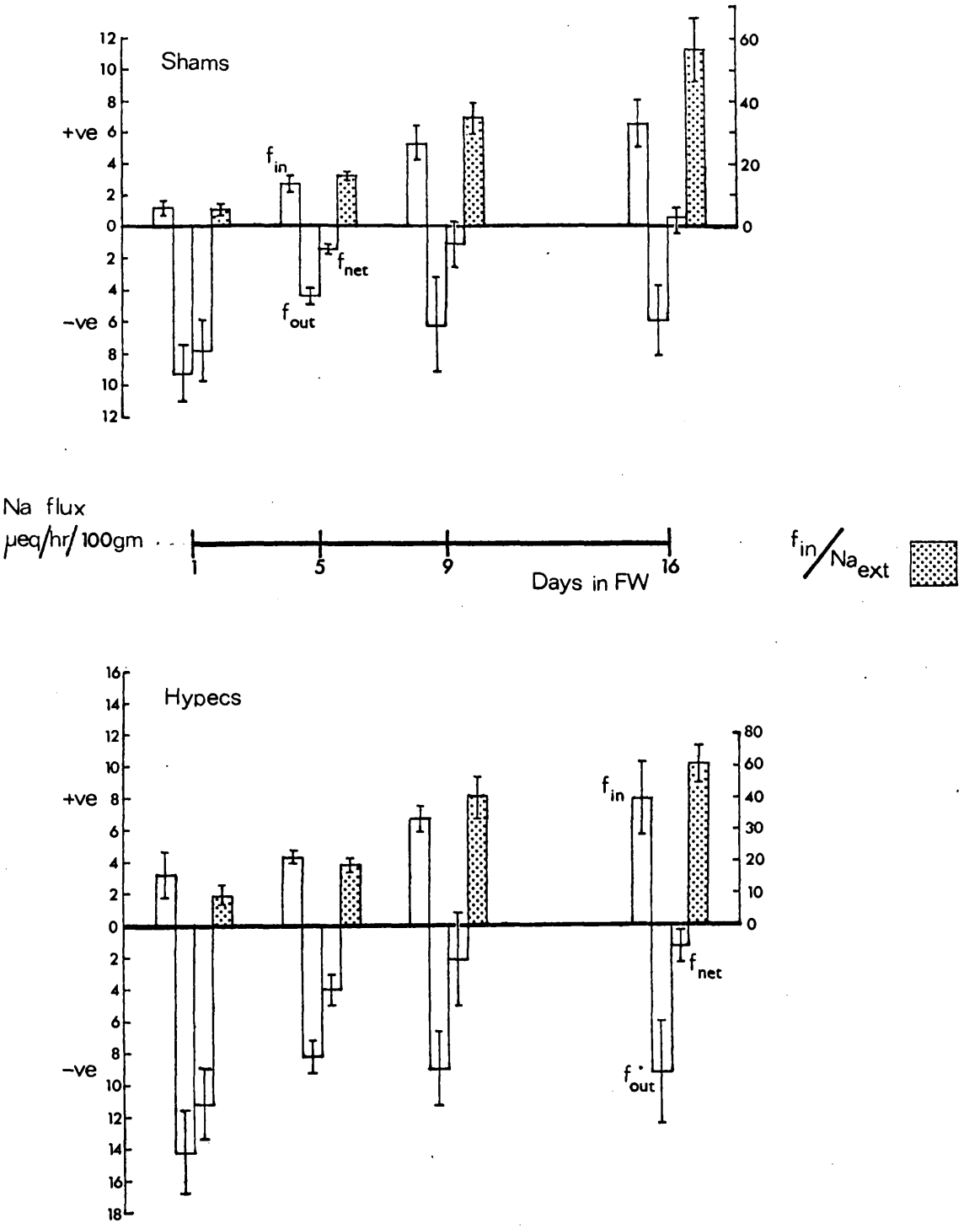
Days		f_{in}	f_{net}	f_{out}	mean Na_{ext}	f_{in}/Na_{ext}
1	S (n = 4)	1.2 ± 0.41	-7.9 ± 1.92	9.1 ± 1.79	0.219	5.7 ± 1.9
	H (n = 6)	3.2 ± 1.27	-11.2 ± 2.16	14.2 ± 2.56	0.298	9.3 ± 2.9
5	S (n = 7)	2.7 ± 0.49	-1.5 ± 0.29	4.2 ± 0.50	0.172	16.0 ± 2.8
	H (n = 7)	4.3 ± 0.37	-4.0 ± 0.85	8.3 ± 0.91	0.236	18.6 ± 1.7
9	S (n = 4)	5.2 ± 1.10	-1.10 ± 1.43	6.3 ± 2.47	0.149	34.3 ± 4.6
	H (n = 4)	6.7 ± 0.80	-2.20 ± 2.87	8.9 ± 2.28	0.168	40.5 ± 6.3
16	S*(n = 3)	6.5 ± 1.50	+0.5 ± 0.62	6.0 ± 2.21	0.116	55.6 ± 10.2
	H*(n = 3)	7.9 ± 2.25	-1.4 ± 1.28	9.3 ± 3.24	0.154	50.1 ± 5.6

S = Sham., H = Hypec., S* = Sham. +6 0.8% NaCl injections, H* = Hypec. + 6 prolactin injections (1µg/gm)

Fluxes - µEq/hr/100 gm, Na_{ext} - mEq/l

Figure 6.

Time course of the FW-adaptation of sodium fluxes (see Table 18).


Shams
+ve
-ve
f_{in}
f_{out}
f_{net}
Na flux
μeq/hr/100gm
1
5
9
16
Days in FW
f_{in}/Na_{ext}
Hypecs
+ve
-ve
f_{in}
f_{out}
f_{net}

to sodium ions was increased leading to a strong negative sodium balance despite a normal sodium absorption mechanism.

Effects of prolactin upon sodium balance

In sea water In Table 19 the mean sodium turnovers of sham-operated flounders adapted to sea water are compared before and after ovine prolactin injections (N1H-PS8, 4 x 5 μg/gm/day). The rate of sodium exchange was reduced substantially ($P < 0.05$) by the prolactin treatment.

In freshwater Ovine prolactin (6 x 5 μg/gm/day) was injected into hypophysectomised flounders held in freshwater with saline-injected intact fish as controls. The results are given in Table 18. On the sixteenth day in freshwater the sodium efflux of ablated fish injected with prolactin did not differ from the value recorded on the ninth day; the influx and net efflux of sodium ions were similarly unaffected.

Tritiated water exchange

The diffusional flow of water across a membrane occurs by the random movements of water molecules in contrast tothe osmotic flow which operates by the bulk transfer of water driven by osmotic forces. The diffusional permeability of the flounder was measured as the rate constant (λ_{HTO}) for the exchange of

Table 19

Effect of prolactin (5μg/gm/day) on sodium turnover (%/hr) of flounders in sea water

Non - injected	After 4 injections prolactin	Change in turnover
43.3 ± 3.10 (n = 4)	24.4 ± 6.02 (n = 4)	-18.9 ± 4.54

Non-injected : injected $P < 0.05$

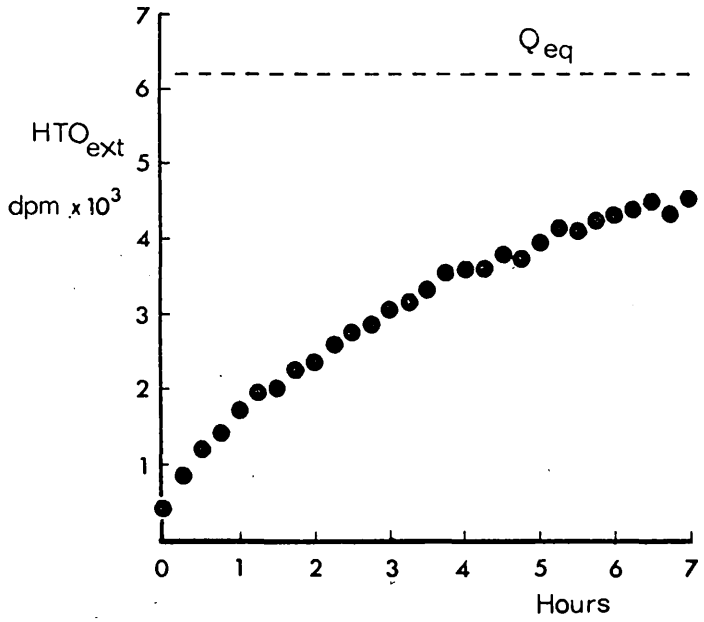
tritiated water (HTO) between the internal water compartment of the fish and the external water. A typical curve for the appearance of injected HTO in the external medium and the subsequent graphical analysis are given in Figure 7 for a sham-hypophysectomised fish adapted to sea water. The value λ_{HTO} was corrected to include the internal water compartment of the fish (70 ml/100 gm, Thorson, 1961; Lahlou and Sawyer, 1969b) to give the turnover in %/hr. The means for HTO turnovers of flounders in various media are given in Table 20. Motais et al (1969) have shown that the gill membrane is the main site of HTO exchange, the contribution of the skin being negligible.

Transfer of flounders from sea water (long term-adapted) to one-third sea water (7 days) and thence to freshwater (< 7 days) was without a significant effect upon HTO exchange. However the rate of exchange in freshwater exceeded that in sea water when the period of hypoosmotic adaptation exceeded seven days. These increases were significant in both sham-operated ($P < 0.05$) and hypophysectomised flounders ($P < 0.01$), but pituitary ablation did not affect HTO exchange.

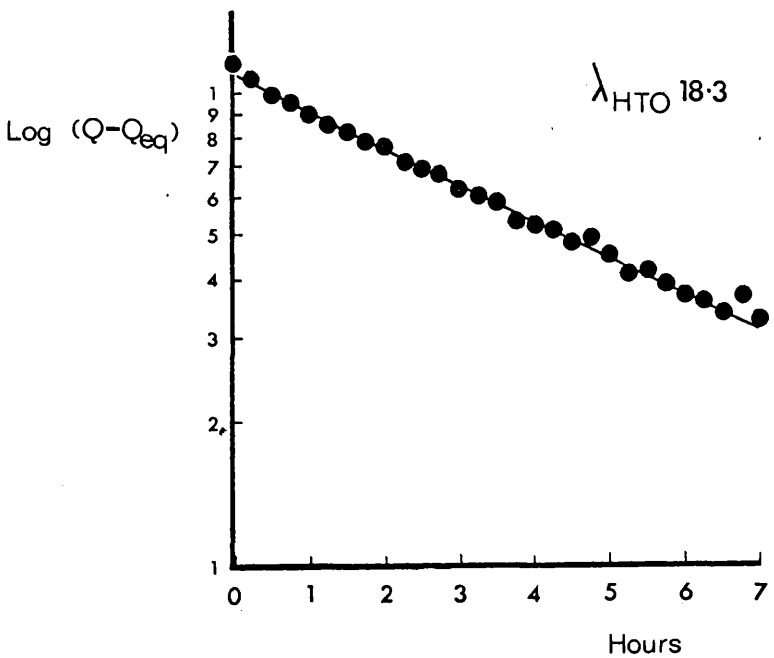
Treatment with ovine prolactin (x 3, x 7 injections 5µg/gm/day) did not affect the HTO exchange rate of hypophysectomised fish in freshwater. As a control sham-operated fish received injections of 0.8% saline. Their HTO turnovers after twelve and fifteen days in freshwater

Figure 7.

(a) HTO evolution curve and (b) semi-logarithmic plot to obtain the HTO turnover, λ_{HTO} (%/hr), for an intact flounder adapted to sea water (see Table 20).


Q_{eq}
HTO_{ext}
dpm x 10^3
7
6
5
4
3
2
1
0 1 2 3 4 5 6 7
Hours

(a)


Log $(Q-Q_{eq})$
λ_{HTO} 18·3
1
9
8
7
6
5
4
3
2
1
0 1 2 3 4 5 6 7
Hours

(b)

Table 20

Tritiated water turnover (λ_{HTO}) and water efflux (f_{out})

	Shams λ_{HTO} %/hr	f_{out} ml/hr/100 gm	Hypecs λ_{HTO} %/hr	f_{out} ml/hr/100 gm
SW	15.4 ± 1.40 (n = 10)	10.8	14.0 ± 1.24 (n = 8)	9.8
1/3 SW	11.6 ± 1.33 (n = 8)	8.1	13.6 ± 1.25 (n = 4)	9.5
FW < 1 week	16.1 ± 1.33 (n = 7)	11.3	15.2 ± 0.76 (n = 3)	11.6
FW > 1 week	23.1 ± 2.23* (n = 5)	16.2	23.1 ± 1.30** (n = 5)	16.2
	+ 0.8% NaCl injections		+ prolactin injections 5 µg/gm/day	
FW 12 days 3 injections	24.3 ± 3.20 (n = 3)	17.0	20.4 ± 0.48 (n = 3)	14.3
FW 15 days 7 injections	22.0 ± 1.88 (n = 3)	15.4	23.9 ± 3.72 (n = 3)	16.7

SW : FW > 1 week *P <0.02 **P <0.005

were similar to the >7 day value indicating that freshwater adaptation was complete.

The present data upon HTO turnover in intact flounders were lower than the values, 19.8%/hr in sea water and 31.9%/hr in freshwater determined by Motais et al (1969). Both investigations were made at 16°C. In the present investigation the turnover rate in freshwater (>7 days) exceeded that in sea water by the same factor (x 1.5) as that noted by Motais et al for the flounder and Anguilla anguilla. It is possible that "shock effects" in the earlier experiments led to an increased water permeability, as has been observed in Carassius auratus (Lahlou and Giordan, 1970). The flounders used in the present experiments would have become accustomed to frequent handling during the preceding surgery and determinations of sodium balance.

The diffusional efflux of water (f_{out}) given in Table 20 was calculated from the mean HTO turnover using the relationship:

$$f_{out} = \lambda_{HTO} \cdot V_i$$

where V_i is the volume of the internal water compartment (70 ml/100 gm) and f_{out} is expressed as ml/hr/100 gm.

Using Fick's Law, the theoretical net flux of water (f_{net}) passing through the permeable membranes of the fish was calculated from the equation:

$$f_{net} = f_{out} \cdot \frac{\triangle c}{a_i}$$

where $\triangle c$ is the osmotic pressure gradient (mOsm/kg)

between the internal and external media, and a_i the internal molar concentration of water (55.5 moles/l). Thus for the sham-operated group of flounders in sea water

$$f_{net} = 10.8 \times \frac{1100 - 330}{55.5} = 150 \text{ µl/hr/100 gm}$$

and similarly in freshwater (*circa* zero mOsm/kg)

$$f_{net} = 16.2 \times \frac{280}{55.5} = 82 \text{ µl/hr/100 gm}$$

These values may be compared with the osmotic net fluxes of water across the branchial membrane calculated by difference from the drinking rate and urine flow by Motais *et al* (1969).

- 145 µl/hr/100 gm in sea water
+ 250 µl/hr/100 gm in freshwater

A comparison of these theoretical and measured net fluxes permits the relationship between the diffusional and osmotic permeabilities to be determined (Lahlou and Giordan, 1970) since the ratio of the two permeability coefficients

$$\frac{P_{osm}}{P_{diff}} \simeq \frac{f_{net}\ \text{measured}}{f_{net}\ \text{theoretical}}$$

This ratio was unity for flounders adapted to sea water and 3.0 for fish in freshwater (>7 days), agreeing closely with the respective values, 0.8 and 2.6 derived by Motais *et al* using a slightly different method.

The aqueous permeability of the gill in sea water was lower than in freshwater despite the greater osmotic gradient of the hyperosmotic medium (SW *circa* 1100 mOsm/kg: flounder 330 mOsm/kg:FW *circa* 1 mOsm/kg). The low permeability persisted under isosmotic conditions in one-third sea water but might have increased with an extended period of adaptation as occurred in freshwater. The diffusional transfer of water was of major importance in sea water, but in freshwater assumed a lesser role by comparison with the osmotic transfer of water.

CHAPTER V

DISCUSSION

EFFECTS OF HYPOPHYSECTOMY ON OSMOREGULATION

In Sea Water

Blood composition

The osmolality and ionic composition of the blood of intact, SW-adapted flounders are comparable with data on this species and various other euryhaline teleosts (Lange and Fugelli, 1965; Chan *et al*, 1967; Lahlou, 1967; Stanley and Fleming, 1964,1967b; Hickman, 1968b; Pickford *et al*, 1969).

Previous investigations on the blood composition of hypophysectomised teleosts have often been restricted to determinations of total osmolality and/or sodium and chloride content since these ions generally contribute *circa* 90% of the osmolality. In the hypophysectomised flounder plasma chloride was reduced slightly. A concomitant reduction in serum osmolality occurred but plasma sodium was not altered by the ablation. A reduction in chloride, independent of sodium, could have led to an imbalanced ionic charge and alkalosis,

but possible changes in serum proteins binding sodium or in another anionic component (probably bicarbonate) might have compensated for the apparent excess of cations. In the SW-adapted Fundulus heteroclitus plasma chloride was reduced following hypophysectomy but plasma sodium was not affected (Pickford et al, 1970a). By contrast, in ablated Fundulus kansae, Anguilla anguilla and Poecilia latipinna sodium levels in the blood were increased (Butler, 1966; Stanley and Fleming, 1967b; Ball and Ensor, 1969), whereas in Anguilla anguilla and Fundulus heteroclitus slight (but non-significant) reductions in sodium levels have been reported elsewhere (Maetz et al, 1967a,b).

Hypophysectomy did not influence the plasma content of potassium, calcium and magnesium in flounders maintained in sea water. Corresponding observations for potassium and calcium have been made in Anguilla anguilla and Fundulus kansae (Butler, 1966; Stanley and Fleming, 1967b) but no other data on magnesium are available.

The haematocrit measurements were incidental to the preparation of serum and plasma samples for analysis but they present evidence of an hormonal deficiency in hypophysectomised flounders. The low haematocrit of intact fish was probably a consequence of the low water temperature (10°C) as has been observed in investigations on other teleosts (Anthony, 1961; Hevesy et al, 1964; Slicher and Pickford, 1968). However the slight aggravation of the anaemia in hypophysectomised flounders maintained in sea water corresponded to the more pronounced reductions in haematocrit observed in ablated Fundulus heteroclitus

and Fundulus kansae (Slicher, 1961; Slicher and Pickford, 1968; Stanley and Fleming, 1967b). In Fundulus heteroclitus a normal haematocrit was restored by ACTH or cortisol treatment (Slicher, 1961; Pickford et al, 1970a). It is possible that the reduced haematocrit in the ablated flounder was mediated by a cortisol deficiency induced in the absence of hypophysial ACTH. This suggestion is in line with others made later concerning the hypophysial control of sodium exchange in SW-adapted flounders and a reduction in plasma cortisol might also be related to the reduced plasma chloride of hypophysectomised fish.

Sodium exchange

The sodium turnover and total sodium efflux of intact flounders adapted to sea water are in close agreement with previous data on this species (Motais et al, 1966; Motais, 1967). The minor contribution (o.1%) of the renal sodium efflux towards the total flux confirms earlier demonstrations by Motais and his co-workers that the main site of sodium exchange is extrarenal, presumably being the gill membrane. In hypophysectomised flounders the sodium turnover and total ion efflux were depressed by one-fifth. In hypophysectomised Anguilla anguilla and Fundulus heteroclitus more extensive reductions (circa 50%) occurred (Maetz et al, 1967a, b) but in ablated Poecilia latipinna this effect was absent despite an increase in internal sodium content (Ball and Ensor, 1969; Ball unpublished, cited Olivereau and Ball, 1970). In hypophysectomised Fundulus kansae a reduction in extrarenal sodium excretion was accompanied by a rise in sodium serum (Stanley and Fleming, 1967b).

The isotopic measurement of the net branchial sodium efflux in SW-adapted flounders is complicated by the presence of the large Na:Na exchange diffusion component (Motais et al, 1966). The net and exchange sodium fluxes are both mediated by the same carrier, the net flux operating by the exchange of internal sodium against external potassium (Motais et al, 1966; Motais, 1967; Maetz et al, 1969a; Maetz, 1969b). Evidence for a close linkage of these two efflux components was obtained in Anguilla anguilla. Transfer to media hypersaline to sea water enhances both the exchange and net fluxes (Maetz and Skadhauge , 1968) and actinomycin D treatment impairs branchial sodium exchange with an accompanying increase in internal sodium indicating a parallel reduction of the net flux (Maetz et al, 1969b). On the assumption that the net branchial efflux compensates intestinal sodium absorption Maetz (1969b) calculated the net flux as 100 μeq/hr/100 gm from the drinking rate of the flounder. This value represents 3.5% of the total branchial efflux. The Na:Na exchange diffusion component contributes 85-90% (Motais, 1967) and it is presumed that the remainder (circa 10%) represents a passive flux of sodium ions across the branchial epithelium.

Since the sodium turnover and efflux of ablated flounders were reduced by 20% it is evident that the exchange diffusion component was depressed. In intact flounders the instantaneous reduction (circa 90%) of sodium efflux at SW→FW transfer was due primarily to the abolition of this component. In hypophysectomised fish the reduced efficiency of the instantaneous regulation provided

additional evidence that pituitary ablation impaired Na:Na exchange diffusion.

As the Na:Na and Na:K exchange fluxes are closely linked it is likely that the efficiency of the branchial sodium pump was also depressed by hypophysectomy. In fact the augmentation of sodium efflux after FW→FW + K^+ transfer mediated by the reappearance of Na:K exchange, was rather less marked in hypophysectomised fish than in their sham-operated controls, indicating an impaired sodium pump.

The Na:K exchange pump is abolished in flounders transferred from sea water into K^+-free sea water (Maetz, 1969b). Assuming a similar effect upon SW→FW transfer (additional to the prevention of Na:Na exchange diffusion) the efflux persisting in freshwater would represent the passive component of sodium efflux. In hypophysectomised flounders this flux was high, indicating an increase in the passive sodium permeability of the branchial membrane. In sea water this effect would tend to be obscured by the high rate of branchial sodium exchange.

In the freshwater teleost hypophysial "prolactin" controls the passive sodium permeability of the gill membrane. Information concerning the role of prolactin in SW-osmoregulation is limited, though the hormone is concerned evidently in the control of sodium exchange in Poecilia latipinna in saline media (Ball and Ensor, 1969). In hypophysectomised flounders the absence of a prolactin-like factor was not responsible for the impaired sodium exchange since injections of ovine prolactin depressed

rather than augmented the sodium turnover of intact fish. Prolactin treatment was ineffective in reversing the reduced sodium exchange of hypophysectomised Fundulus heteroclitus and Anguilla anguilla (Maetz et al, 1967a,b).

Observations on other teleosts indicate strongly than an abolition of ACTH secretion and a resultant cortisol deficiency were responsible for the impaired sodium exchange of hypophysectomised flounders. Treatment with ACTH restored the reductions in sodium turnover completely in hypophysectomised Anguilla anguilla and partially in ablated Fundulus heteroclitus (Maetz et al, 1967c; Maetz, 1969a; Mayer, 1970). These effects correlate with the severe reduction of sodium turnover in adrenalectomised Anguilla anguilla and its complete reversal by cortisol injections. Sodium efflux was impaired by adrenalectomy, resulting in a rise in internal sodium and subsequent death. Cortisol treatment repaired these defects to permit a regulated sodium balance and prolonged survival (Chan et al, 1967; Mayer et al, 1967; Maetz, 1969a). The effects of hypophysectomy upon sodium exchange are considerably less severe than those of adrenalectomy. In the flounder and other teleosts maintained in sea water alterations in plasma sodium following hypophysectomy are either slight or non-existent and survival is not impaired. The main source of endogenous corticosteroids (predominantly cortisol) is removed by adrenalectomy but the absence of adrenocorticotrophic stimulation in hypophysectomised fish would tend to inhibit but not necessarily abolish steroid secretion. Previous work upon Anguilla spp. has shown that a marked

reduction in plasma cortisol following hypophysectomy may be restored to normal by ACTH treatment (Bradshaw and Fontaine-Bertrand, 1968; Butler *et al*, 1969a; Hirano, 1969; Hawkins and Ball, 1970; Ball *et al*, 1971). In hypophysectomised *Anguilla anguilla* plasma cortisol was diminished but did attain stable levels (Butler *et al*, 1969a).

The reduction in branchial sodium exchange in the hypophysectomised flounder was less marked than in *Fundulus heteroclitus* and *Anguilla anguilla* (Maetz *et al*, 1967a,b) and plasma sodium levels remained constant in the three teleosts. However increases in internal sodium in ablated *Fundulus kansae* (Stanley and Fleming, 1967b) and *Anguilla anguilla* (Butler, 1966) were attributed to an impaired branchial sodium excretion. The maintenance of a constant plasma sodium despite a reduced branchial ion efflux would require compensatory adjustments in either intestinal or renal sodium fluxes. In the SW-adapted flounder the renal sodium efflux is minute before the net branchial efflux, and, as in the eel and *Fundulus kansae*, this parameter was not altered by hypophysectomy. Therefore it is probable that pituitary ablation also reduced intestinal sodium absorption in the flounder. In SW-adapted *Anguilla japonica* hypophysectomy depressed intestinal sodium exchange; this effect was reversed by ACTH and cortisol treatment (Hirano, 1967; Hirano *et al*, 1967; Hirano and Utida, 1968). A similar action of cortisol has been confirmed recently in *Anguilla anguilla* (Payan unpublished, cited Maetz, 1970c).

Studies upon the enzyme Na + K-ATPase implicated in

sodium transport activity in teleost osmoregulatory organs provide corroborative evidence that the ACTH-corticosteroid axis exerts a major influence over the sodium exchange of teleosts in sea water (Utida et al, 1966; Epstein et al, 1967, 1971; Pickford et al, 1970a; Milne et al, 1971). The effects of hypophysectomy and ACTH or cortisol treatment upon intestinal and branchial Na + K-ATPase are in parallel with their effects upon sodium transport in these organs. In SW-adapted Fundulus heteroclitus hypophysectomy reduced the branchial Na + K-ATPase content and cortisol treatment partially reversed this effect; the hormone also stimulated the intestinal enzyme content (Pickford et al, 1970a). In Anguilla anguilla branchial Na + K-ATPase was reduced by pituitary ablation and restored to normal levels by ACTH treatment (Milne et al, 1971). The tendency for hypophysectomy and ACTH/cortisol to influence sodium exchange and/or Na + K-ATPase content at both the gill and intestine lends support to the view that the depression of branchial sodium exchange in the ablated flounder was accompanied by a corresponding effect at the intestine.

Urine flow and composition

In SW-adapted intact flounders the rates of urine flow were lower than in previous measurements made by different techniques (Henschel, 1936; Lahlou, 1967; Motais, 1967). In the present investigation the low water temperature (10°C) may have influenced urine flow since Lahlou and Motais used a warmer environment (16-18°C). In SW-adapted Paralichthys

lethostigma a seasonal variation in urine flow could be attributed to a 10°C temperature difference (Hickman, 1968a). In the freshwater teleost, Catostomus commersonii (white sucker) a similar temperature-dependent (2 → 18°C) increase in urine flow was related to an enhanced surface permeability to water (Mackay and Beatty, 1968). In Anguilla anguilla an increased environmental temperature raised the surface water permeability (measured as HTO turnover, Motais and Isaia, 1970 unpublished). However a corresponding change in the surface permeability of the flounder adapted to sea water would lead to reduction in urine flow to compensate a higher osmotic efflux of water.

The osmolality and sodium content of the urine of intact flounders in Experiment I are comparable with other data on these parameters (Lahlou, 1967), but both urine osmolality and sodium were increased considerably in Experiment II. In teleost/ean urine magnesium predominates over sodium in SW-adapted fish for divalent ion excretion is a major function of the kidney (Hickman and Trump, 1969). These ionic concentrations were reversed in the urine of the flounder. The low urine flow provides a possible explanation for this observation, since at comparable flow rates in Paralichthys lethostigma and Anguilla anguilla urine sodium increased to displace magnesium as the major cation (Hickman, 1968b; Chester-Jones et al, 1969b). The potassium, calcium and chloride levels in the urine of SW-adapted flounders are comparable with data on the flounder and other euryhaline teleosts (Sharrat et al, 1964a; Lahlou, 1967; Hickman, 1968b).

Chloride is the major anion in the urine of marine teleosts.

The ability to secrete a hyperosmotic urine has not been reported previously in the flounder. As in other teleosts (Fundulus kansae, Stanley and Fleming, 1964, Fleming and Stanley, 1965; Paralichthys lethostigma, Hickman, 1968b) this phenomenon appeared to be associated with experimental procedures involving stress. It is suggested that the technique for urine flow measurement provoked a natriuresis and, in consequence, the frequent secretion of plasma-hyperosmotic urines. In catheterized flounders an abolition of bladder sodium reabsorption (Lahlou, 1967) would prove an additional factor enhancing urine osmolality. In SW-adapted Paralichthys lethostigma the secretion of a hyperosmotic urine was mediated by a natriuresis following bladder catheterization (Hickman, 1968b).

It is interesting to speculate on the possibility that endocrine mechanisms mediate the formation of a hyperosmotic urine. The role of stress effects in the phenomenon suggests that adrenocortical factors are implicated. In SW-adapted Anguilla anguilla shock-induced increases in sodium exchange have been correlated with analogous effects produced by ACTH or cortisol treatment (Mayer and Maetz, 1967; Maetz, 1969a; Mayer, 1970). In the eel a transitory rise in plasma cortisol was associated with the stress of handling during SW→SW transfer (Ball et al, 1971). In hypophysectomised Fundulus heteroclitus maintained in sea water cortisol treatment altered the renal activity of the sodium

transport enzyme Na + K-ATPase (Pickford et al, 1970a). A plasma cortisol deficiency arising from abolition of ACTH secretion could explain the virtual absence of hyperosmotic urine in hypophysectomised flounders. In Fundulus kansae pituitary ablation has a similar effect in preventing the secretion of a hyperosmotic urine (Stanley and Fleming, 1966a,b), however the mechanism responsible appears to differ in the two teleosts. In ablated Fundulus kansae the marked natriuresis following FW→SW transfer was reduced but in the flounder the decline in urine osmolality seemed to be mediated by a reduction in urine chloride, with sodium levels remaining similar to those in intact fish. These observations suggest that a natriuretic effect is not the main factor mediating a plasma-hyperosmotic urine, in contrast to the inference made from the data upon sodium in intact flounders. The low urine chloride of hypophysectomised fish was probably related to the reduction in plasma chloride, indicating an attempt to conserve this anion. No significant reduction in renal chloride efflux was apparent due to the highly variable data. Evidently the tubular reabsorption of chloride was increased markedly since the urine flow and renal sodium efflux were not altered. In the intact flounder 80% of the chloride in the glomerular filtrate was subsequently reabsorbed in the tubule (Lahlou, 1967).

The sodium, potassium and magnesium levels in the urine were not affected by hypophysectomy but urine calcium was reduced slightly. In teleosts calcium ions

are more abundant in urine than in plasma since the former is the major route for the excretion of calcium absorbed from ingested sea water. Urine calcium ions arise by active tubular secretion and presumably hypophysectomy depressed this mechanism. In SW-adapted Anguilla anguilla urine flow and electrolytes (Na^+ and K^+) were not affected by hypophysectomy (Butler, 1966), and in Fundulus kansae adaptation of urine flow and sodium excretion after FW→SW transfer was less efficient but this was only a temporary effect (Stanley and Fleming, 1966a,b,1967b).

The renal electrolyte fluxes were low in intact, SW-adapted flounders: that for sodium is comparable with earlier data (Lahlou, 1967; Motais, 1967). The fluxes were not influenced by hypophysectomy, with the exception of the calcium efflux which was reduced.

In conclusion further investigation is required before the mechanisms involved in the secretion of a hyperosmotic urine can be elucidated. Meanwhile the physiological significance of the phenomenon is open to conjecture and it is possible to draw only tentative conclusions concerning the hypophysial control of osmoregulation at the renal level in the flounder.

Water exchange

In flounders maintained in sea water (and one-third sea water) tritiated water turnover was not affected by hypophysectomy, indicating that the aqueous permeability of the gill, the major site of HTO exchange (Motais et al, 1969), is not under hypophysial control. Since drinking

rates were not determined, reliable estimates of the net osmotic water fluxes could not be made. However it is probable that the osmotic water permeability was not affected by hypophysectomy since the plasma electrolyte levels (with the exception of slight reduction in chloride) and urine flow were normal in ablated fish. In hypophysectomised Fundulus kansae total body water and urine flow were normal (Stanley and Fleming, 1967a) and evidence of a slight reduction in water permeability has subsequently been detected in HTO turnover determinations (Potts and Fleming, 1970).

In Freshwater

Blood composition

The decline of circa 20% in the osmolality and electrolyte content of the blood of intact flounders eleven days after SW→FW transfer is compatible with other data on this species (Lange and Fugelli, 1965; Lahlou, 1967; Motais, 1967) and various euryhaline teleosts following freshwater adaptation (Salmo trutta, Gordon, 1959; Mugil cephalus, McFarland, 1965; Anguilla anguilla, Chan et al, 1967; Fundulus kansae, Stanley and Fleming, 1967b). The reductions in plasma sodium and chloride ions were sufficient to account for the difference in serum osmolality; the contribution from slight (but significant) alterations in the other plasma ions determined was negligible.

In hypophysectomised flounders a slight aggravation of the progressive reductions in serum osmolality

associated with normal freshwater adaptation became apparent after only two days in the hypoosmotic medium. By the eleventh day the osmolality, and sodium and chloride content of the blood in the ablated fish were reduced considerably below the levels recorded in intact fish. As in the intact flounders the total reduction of 30% in the serum osmolality of hypophysectomised fish was due primarily to a decreased plasma sodium and chloride. This inability of the hypophysectomised flounder to maintain normal levels of blood sodium and chloride in freshwater corresponds to similar observations on various stenohaline and euryhaline teleosts, notably Fundulus heteroclitus (Burden, 1956, Pickford et al, 1966a), Fundulus kansae (Stanley and Fleming, 1967b), Anguilla anguilla and A. rostrata (Chester Jones et al, 1965; Butler, 1966,1967; Olivereau and Chartier-Baraduc, 1966), Tilapia mossambica (Dharmamba et al, 1967), Poecilia latipinna (Ball and Ensor, 1965,1967) and Carassius auratus (Donaldson et al, 1968; Lahlou and Sawyer, 1969a).

Among teleostean species pituitary ablation exerts variable effects upon the ability to survive in freshwater (see Table 1). Many teleosts, particularly members of the Order Antheriformes are unable to tolerate freshwater following hypophysectomy (Pickford, 1953; Ball and Olivereau, 1964; Jalabert and Billard, 1968; Schreibman and Kallman, 1966,1969). An impaired survival has been attributed directly to a defective electrolyte balance, and maintenance in full or dilute sea water, or teleostean Ringer permits survival and facilitates a recovery from "osmotic shock" (Burden, 1956; Pickford et al, 1965; Ball and

Olivereau, 1964; Ball and Ensor, 1965,1967; Schreibman and Kallman, 1965,1966). Hypophysectomised teleosts belonging to other orders may behave similarly to the majority of Antheriform species, for example Tilapia mossambica and Betta splendens Perciformes (Handin et al, 1964; Schreibman and Kallman, 1965; Dharmamba et al, 1967), or alternatively, they may survive in freshwater indefinitely despite reductions in plasma electrolytes, as in Anguilla anguilla Anguilliformes, Carassius auratus Cypriniformes, Salmo gairdnerii Salmoniformes (Fontaine et al, 1949; Olivereau and Fontaine, 1965; Butler, 1966; Chavin, 1956; Olivereau and Chartier-Baraduc, 1966; Donaldson and McBride, 1967; Lahlou and Sawyer, 1967,1969a).

No previous reports concerning the effects of hypophysectomy on osmoregulation in the Order Heterosomata, the flatfishes, exist in the literature. Barr (1963) used this technique to study the endocrine control of reproduction in the marine (stenohaline) plaice, Pleuronectes platessa. Ablated plaice survived well in sea water, but only in the months outwith the spawning period (July - November). Hypophysectomised flounders survived indefinitely in sea water but spent fish caught in May were less tolerant to freshwater after hypophysectomy than fish collected during October - November. It is probable that the survival of hypophysectomised flounders in freshwater (sixteen days as against three weeks for intact fish) would have been extended if the fish had been fed and not subjected to the shock effects of blood and urine sampling tending to aggravate sodium depletion. Among the

intact flounders an accidental rise in water temperature was another factor which caused premature mortalities. Thus the observed effect of hypophysectomy in impairing freshwater tolerance in the flounder should be interpreted with caution; it would be profitable to repeat the experiment using sham-operated and ablated flounders maintained under more equable conditions. Survival periods reported for hypophysectomised Anguilla anguilla under different experimental conditions ranged from under four weeks (Butler, 1966) to over eighteen months (Olivereau and Fontaine, 1965). Despite a rigo rous experimental regime the mean survival period of the hypophysectomised flounder exceeded the periods of 6-7 days in Fundulus heteroclitus (Burden, 1956), 1-2 days in Poecilia latipinna (Ball and Ensor, 1969), 6-8 days in Tilapia mossambica (Handin et al, 1964) and circa 10 days in Xiphophorus spp. (Schreibman and Kallman, 1966,1969).

Typical reductions (below control levels) in the osmolality and sodium/chloride content of the blood of hypophysectomised teleosts subsequently dying in freshwater included: 40-60%, osmolality and chloride in Fundulus heteroclitus (Burden, 1956; Pickford et al, 1965), 25%, sodium in Poecilia latipinna (Ball and Ensor, 1965) and 45%, osmolality in Tilapia mossambica (Dharmamba et al, 1967). By contrast the reductions of 13% in serum osmolality and 17-18% in plasma sodium chloride recorded in the ablated flounder were less severe, being akin to those noted in species tolerant to freshwater, 18%, sodium in Anguilla anguilla (Olivereau and Chartier-Baraduc, 1966) and 10%,

osmolality and sodium in Fundulus kansae (Stanley and Fleming, 1967b). An ability to withstand a considerable dilution of the internal media is also important in freshwater tolerance since in ablated Carassius auratus 33% reductions in plasma sodium and chloride have been recorded (Lahlou and Sawyer, 1969a).

Equivocal results concerning the decline in plasma potassium on freshwater adaptation were obtained for the intact flounders since the consistent reductions in plasma sodium (18-19%) were not matched by those for potassium (controls, 9%; shams, 17%). Lahlou (1967) noted corresponding reductions (13-14%) in the two cations, in contrast to Lange and Fugelli (1965), who found that the reduction in sodium (17%) exceeded that for potassium (6%). Variable differences between the two ions on freshwater adaptation have also been reported in other euryhaline teleosts, for example, Anguilla anguilla (Sharrat et al, 1964a;Butler, 1966), Salmo gairdnerii (Holmes and McBean, 1959; Houston, 1959) and Fundulus heteroclitus (Pickford et al, 1969). The plasma potassium of FW-adapted flounders was not influenced by hypophysectomy and the possible trend towards a decline in the plasma Na^+/K^+ in the ablated fish was a consequence of the aggravated reduction in plasma sodium. A similarly induced reduction in the Na^+/K^+ ratio has been observed in hypophysectomised Fundulus kansae (Stanley and Fleming, 1967b) but in Anguilla anguilla a reduced Na^+/K^+ was mediated by a decline in plasma sodium and a simultaneous rise in potassium (Olivereau and Chartier-Baraduc, 1966;

Chan et al, 1968). In some intact teleosts (Mugil cephalus, Anguilla anguilla) an inverse relationship exists apparently between the sodium and potassium content of the body fluids, a reduction in blood sodium on FW-adaptation being accompanied by a rise in potassium probably originating from an intracellular source (McFarland, 1965; Chester Jones et al, 1965; Stanley and Fleming, 1967b). Both the present and earlier investigations (Lange and Fugelli, 1965; Lahlou, 1967) indicate that this mechanism is not operative in the flounder, even under the provocation of a marked decline in plasma sodium in hypophysectomised fish.

The plasma calcium of intact FW-adapted flounders was circa 4 meq/1, slightly lower than the value of 5.37 meq/1 determined by Lahlou (1967). No published values for plasma magnesium in this species are available and the value of circa 0.8 meq/1 was considerably lower than in other euryhaline teleosts (3.3 meq/1 Fundulus heteroclitus, Pickford et al, 1969; 5.22 meq/1 Anguilla anguilla, Chan et al, 1968; 2.53 meq/1 Anguilla rostrata, Butler et al, 1969b). Plasma calcium was not affected by hypophysectomy, but magnesium was reduced markedly. Few studies of the effects of hypophysectomy on divalent ion regulation have been made, and those data available are equivocal. In ablated Fundulus kansae serum calcium was increased slightly (Stanley and Fleming, 1967b), but in Anguilla anguilla calcium was reduced along with the other plasma electrolytes (Olivereau and Chartier-Baraduc, 1966; Chan et al, 1968; Chan and Chester Jones, 1968). The reduction in plasma magnesium in the flounder contrasts to the slight increase observed in ablated Fundulus heteroclitus (Pang, 1969) and

the absence of any effect in Anguilla anguilla (Chan et al, 1968). It is possible that a cortisol deficiency due to the abolition of ACTH secretion in the ablated flounder mediated the reduction in plasma magnesium, since in adrenalectomised Anguilla anguilla both serum sodium and magnesium were reduced (Chan et al, 1969) and in similarly treated Anguilla rostrata a unilateral reduction in magnesium was correlated with a decreased plasma cortisol (Butler et al, 1969b). Chronic cortisol treatment raised serum magnesium in hypophysectomised Fundulus heteroclitus, but this effect was observed in sea water (Pang, 1969; Pickford et al, 1970a).

Urine flow and composition

Renal adaptation to freshwater was incomplete after two days in the hypoosmotic medium since further reductions in the osmolality and sodium content of the urine were evident after nine days. The urine flow, the major ions, sodium and chloride, and magnesium were not affected by hypophysectomy, but potassium and calcium levels were increased. The effect upon potassium was seen also in the high renal efflux of this ion, but due to the high variability of the data the renal calcium efflux was not increased significantly in the ablated fish. Since plasma potassium and calcium were not affected by hypophysectomy the high urine levels of these ions are difficult to explain. The high potassium efflux might represent an attempt to reduce plasma potassium and restore the plasma Na^+/K^+ ratio to normal levels, thus supporting earlier indications for the absence of an inverse relationship

between plasma sodium and potassium regulation. Alternatively the haemolysis observed in one plasma sample from a hypophysectomised flounder could have occurred more frequently and have necessitated a high renal ion efflux for the maintenance of a normal plasma potassium. In hypophysectomised Anguilla anguilla a transient increase in renal potassium efflux accompanied a reduced plasma potassium (Butler, 1966). By contrast in ablated Fundulus kansae renal potassium efflux was normal but, as in the flounder, calcium excretion was increased (Stanley and Fleming, 1967b).

The renal sodium efflux of intact FW-adapted flounders (3.5 - 4.0 meq/hr/100 gm) corresponds to other data on this species (5 - 6 meq/hr/100 gm, Lahlou, 1967; Motais, 1967). The increase in urine flow in the hypo-osmotic environment was not compensated fully by the enhanced reabsorption of sodium since the renal sodium efflux was slightly higher than in sea water. A similar observation has been made in Anguilla anguilla (Sharrat et al, 1964a). The renal effluxes of sodium and chloride were not affected by hypophysectomy and therefore made no contribution towards the depletion of these ions evident in ablated fish. This indirect evidence for an enhanced extrarenal sodium efflux in ablated flounders was confirmed later by isotopic measurements on the branchial sodium fluxes. As with other teleostean species so far investigated, the flounder differs from Fundulus kansae, in which sodium depletion following hypophysectomy is mediated by a high renal ion efflux, branchial sodium efflux not being affected (Stanley and Fleming, 1966a,b,1967b).

In ablated Carassius auratus urine sodium was increased but a reduction in urine flow held renal sodium efflux at constant levels (Lahlou and Sawyer, 1969a).

Sodium exchange

The sodium fluxes of flounders adapted to freshwater (external sodium < 0.2 meq/1) correspond to the values obtained by Motais (1967) using water of a similar sodium content. It was apparent that the main adaptation of efflux to freshwater was completed with one day of SW→FW transfer, an efficiency comparable with that of Anguilla anguilla and exceeding that of Fundulus heteroclitus (Maetz et al, 1967a,b). In the flounder sodium influx is dependent on the ion content of the external medium; the active component attains a saturation value of circa 20 μeq/hr/100 gm in approximately 0.2 meq/1 external sodium, in excess of which the linear increase in influx is due to a passive component (Maetz and Zwingelstein unpublished, cited Maetz, 1970a). Ideally sodium fluxes should be measured in fish fitted with a bladder catheter so that renal and extrarenal fluxes may be resolved (Maetz et al, 1967a; Lahlou and Sawyer, 1969a). However in the present investigation the sporadic release of urine could be detected with ease to permit independent estimations of the extrarenal fluxes. The mean renal component of 4.0 μeq/hr/100 gm corresponded with the earlier, direct measurement (3.5 - 4.0 μeq/hr/100 gm) and was 50% lower than the extrarenal flux (or one-third of the total efflux, in agreement with Motais, 1967). The minor role of the renal flux was also demonstrated in Fundulus heteroclitus

(Maetz et al, 1967a) and Carassius auratus (Lahlou and Sawyer, 1969a). It is presumed that the main site of the extrarenal flux was the branchial membrane, though a minor contribution of passive sodium fluxes across the skin could not be discounted definitely. In teleosts the surface area of the skin is small relative to that of the branchial epithelium (Parry, 1966).

The majority of the intact flounders exhibited a negative net branchial sodium efflux, but a net gain was demonstrated in some fish after longer periods (9 and 16 days) of FW-adaptation. Freshwater teleosts maintain an equilibriated electrolyte (sodium) balance in the long term, whilst alternating between positive and negative net fluxes in the short term. Experimental stresses may provoke electrolyte depletion (Lahlou and Sawyer, 1969a) necessitating acclimation to handling or "training" (Slicher et al, 1966; Pickford et al, 1969). Presumably the flounders had become accustomed to handling during earlier experiments, but even so the technique for flux measurement entailed only a minimal disturbance. A positive sodium balance (or at least a steady decline in sodium depletion) was achieved by a progressive enhancement of sodium influx. The marked rise in the ratio $^{f}in/Na_{ext}$ with FW-adaptation is indicative of the increased efficacy of the branchial sodium absorption pump. It has been proposed that sodium uptake at the gill of freshwater teleosts operates by the exchange of internal NH_4^+ (arising from protein metabolism) against external Na^+, internal HCO_3^- being exchanged against external Cl^-, but the carrier mechanism mediating this exchange

has not been resolved (Garcia Romeu and Maetz, 1964; Maetz and Garcia Romeu, 1964; Maetz, 1964, 1970a,b). Evidently the activity of the ionic carrier was enhanced on adaptation to freshwater, presumably in response to internal sodium depletion. Similar increases in sodium absorption have been induced by maintaining teleosts in ion-free water or hypotonic loading (Maetz, 1964; Bourguet, Lahlou and Maetz, 1964; Henderson and Chester Jones, 1967).

In hypophysectomised flounders (adapted to freshwater for five days) the branchial sodium efflux was increased by 100%. Branchial sodium absorption, considered either as an absolute flux or in relation to external sodium, was not affected by pituitary ablation. The resultant aggravation (circa 200%) of the negative net sodium flux can be correlated with marked reductions in plasma sodium observed previously in ablated flounders. Hypophysectomy acted solely at the branchial level since no effect upon renal sodium efflux was detected. This effect of pituitary ablation corresponds to an increase in the passive sodium permeability of the branchial membrane, presumably coupled with a similar effect for chloride since plasma chloride is also diminished in ablated flounders.

Corresponding effects of hypophysectomy increasing extrarenal sodium efflux have correlated with a reduced blood sodium level in Fundulus heteroclitus (Potts and Evans, 1966, Maetz et al, 1967a), Anguilla anguilla (Maetz et al, 1967b), Carassius auratus (Lahlou and Sawyer, 1969a), Poecilia latipinna (Ensor and Ball, 1968a) and Tilapia

mossambica (Dharmamba unpublished, 1969). The flounder is similar to Poecilia latipinna and Anguilla anguilla in that sodium influx was not reduced by pituitary ablation, in fact the flux was augmented slightly in the eel. By contrast, in ablated Fundulus heteroclitus and Tilapia mossambica, the efficiency of branchial sodium absorption was reduced substantially. In the flounder the ablation did not reduce the affinity of the absorption pump for external sodium ions, and the progressive increase in f_{in}/Na_{ext} with freshwater adaptation was identical to that in the intact fish.

The increase in sodium efflux and the resultant sodium depletion (in μeq/hr/100 gm) in the hypophysectomised flounder was more severe than in Anguilla anguilla (in which the higher efflux was only apparent in an external sodium level limiting an equilibriated ion balance in intact fish, Maetz et al, 1967b) but was considerably less so than in Fundulus heteroclitus, Poecilia latipinna and Tilapia mossambica. In these teleosts the magnitude of this impairment in branchial sodium exchange is in parallel with the extent of the decline in plasma sodium following hypophysectomy.

The pattern of instantaneous and secondary reductions in sodium efflux on SW→FW transfer were similar between intact and hypophysectomised flounders, but the extent of initial reduction was lessened by the ablation, thereby provoking an immediate aggravation of sodium efflux. No such effect has been observed in ablated Anguilla anguilla (Maetz et al, 1967b). In intact Fundulus heteroclitus the

instantaneous reduction in efflux is less efficient than in the flounder and Anguilla anguilla due to the reduced role of exchange diffusion in this species (Motais et al, 1966). A reduction in the branchial sodium permeability of ablated Fundulus heteroclitus is still apparent and the sodium efflux is comparable with that of intact fish after one day in freshwater, the enhanced efflux appearing subsequently (Maetz et al, 1967a). Hypophysectomy did not influence the pattern of instantaneous and delayed regulation in Anguilla anguilla (Maetz et al, 1967b). It has been suggested previously that secondary regulation in the flounder to reduce passive branchial sodium permeability is under endocrine control (Motais et al, 1966; Motais, 1967). It is now apparent that the instantaneous phase of regulation is also under endocrine influence, namely by a hypophysial hormone(s). A clear distinction between the secondary regulation of sham-operated and ablated flounders was not apparent with the small numbers of fish used.

Water exchange

The water permeability of both euryhaline and stenohaline teleosts is greater in freshwater than in sea water (Potts et al, 1967; Evans, 1969; Motais et al, 1969; Potts and Fleming, 1970). In the flounder tritiated water exchange increased progressively during freshwater adaptation and attained stable levels after one week, whereupon the rate exceeded that in sea water by a factor (x 1.5) identical to that observed by Motais et al (1969). The intervention of shock effects to enhance water permeability

(Lahlou and Giordan, 1970) may explain the higher HTO turnovers reported by Motais et al, but this suggestion is tentative and further investigation is required.

The HTO turnover of flounders maintained in freshwater was not affected by hypophysectomy, indicating that the water permeability is not under hypophysial control; urine flow was similarly unaffected implying a normal osmotic permeability. It is clear that the reductions in plasma electrolytes observed in ablated flounders can be attributed solely to an impairment of ionic exchanges, that is, a high passive branchial efflux of sodium (and chloride) ions, rather than to an increased influx of water from the hypoosmotic environment. Similar conclusions have been drawn for ablated Fundulus kansae and Carassius auratus, two teleosts in which water permeability and urine flow were reduced (Stanley and Fleming, 1967b; Lahlou and Sawyer, 1969b; Lahlou and Giordan, 1970; Potts and Fleming, 1970). Both diffusional and osmotic permeabilities were reduced in the goldfish and a reduced urine flow was indicative of a similar effect on osmotic permeability in Fundulus kansae though the drinking rate of ablated fish was not determined. In both species these effects were mediated primarily by a prolactin deficiency since hormone treatment restored a normal HTO turnover and urine flow (Stanley and Fleming, 1967a; Lahlou and Giordan, 1970; Potts and Fleming, 1970). By contrast prolactin treatment did not enhance the HTO turnover in a small group of ablated flounders, a result not unexpected since hypophysectomy did not influence this parameter.

Potts and Fleming (1970) found that the water permeability of Fundulus kansae is under the dual control of prolactin and external calcium ions, the latter influencing water exchange by their direct effects on membrane permeability. The effects of calcium and prolactin are antagonistic; in SW-adapted fish a low prolactin secretion and high external calcium combine to reduce permeability, whereas in freshwater a high prolactin and low calcium enhance permeability. The reduced permeability of hypophysectomised Fundulus kansae is due to a prolactin deficiency, but in freshwater a low external calcium promotes an increased permeability but to a lesser extent than in intact fish. In Carassius auratus both ACTH and cortisol also stimulate water exchange (Lahlou and Giordan, 1970). These hormonal effects evident in other teleosts indicate that the inactivity of hypophysectomy and prolactin in influencing water exchange in the flounder may require further investigation.

Prolactin

In sea water

In intact flounders adapted to sea water treatment with ovine prolactin produced a marked depression in sodium turnover. In SW-adapted Fundulus heteroclitus a similar dose of the hormone had a corresponding effect (Maetz et al, 1969a) and in Tilapia mossambica a high dose reduced sodium turnover from 28%/hr to 3%/hr (Dharmamba unpublished, 1969). By contrast prolactin did not affect the turnover or total efflux of sodium in Anguilla anguilla (Mayer, 1970).

In the flounder prolactin depressed branchial sodium exchange; any effect on the low renal efflux is assumed to be negligible. The magnitude of the reduction in sodium turnover indicated that the exchange diffusion component of sodium efflux was depressed. It is probable that the activity of the sodium excretion pump was also diminished due to the close linkage of the two efflux components (Maetz et al, 1969a) but plasma sodium determinations were required to prove this point. In both Tilapia mossambica and Fundulus kansae (in 0.4M saline) internal sodium levels were increased by prolactin treatment (Dharmamba unpublished, 1969; Stanley and Fleming, 1967a). The effect of prolactin on sodium exchange in SW-adapted teleosts can be correlated with its action in reducing the branchial activity of sodium transport enzyme, Na + K-ATPase, in Fundulus heteroclitus (Pickford et al, 1970b).

A sensitivity to the mammalian hormone, prolactin, the teleostean homologue of which is closely implicated in osmoregulation, has not been demonstrated previously in the flounder. The positive response obtained is interesting in view of recent work by Chadwick (1970) on the pituitary gland of the flounder. Chadwick detected a hypophysial factor that could be identified as teleostean prolactin, or the "paralactin" of Ball (1965c). This factor has a similar electrophoretic mobility to tetrapod prolactins and, in the pigeon crop bioassay, elicits a response typical of prolactin extracts from other teleosts (Chadwick, 1966,1970; Nicoll et al, 1966; Nicoll and Bern, 1968). Injections of the flounder prolactin fraction

produced a thickening and folding of the crop-sac mucosa, a response lacking only the fatty granules induced by the mammalian hormone.

At present it is not possible to discern the role of prolactin in the osmoregulation of SW-adapted teleosts. The prolactin-secreting eta cells in the adenohypophysis of euryhaline teleosts are more active in freshwater than in sea water (Ball and Olivereau, 1964; Olivereau and Ball, 1964; Ball and Pickford, 1964; Olivereau, 1966a,1968; Emmart, 1969; Sage, 1966; van Overbeeke and McBride, 1967; Ball and Fleming, 1968; Dharmamba and Nishioka, 1968; Hopkins, 1969) though their inhibition in the marine environment is not complete (Ensor and Ball, 1968b; Olivereau, 1969a; Ball and Ensor, 1969; Ball unpublished, cited Olivereau and Ball, 1970). The effects of exogenous prolactin in the flounder and other teleosts indicate that a high rate of prolactin secretion would be disadvantageous under marine conditions, leading to an accumulation of internal sodium mediated by reductions in the branchial ion fluxes. It is evident that the impaired sodium exchange of hypophysectomised flounders maintained in sea water was not due to a prolactin deficiency, and the relevance of hypophysial ACTH in this concern has been discussed already. Chadwick (1970) was unable to find any difference in hypophysial prolactin activity between flounders in sea water and freshwater but used only a single adaptation period (8 hr). Variations on this period might provide detectable changes in hormone activity.

In freshwater

In the present investigation no attempt was made to assess the action of prolactin on the impaired survival of hypophysectomised flounders maintained in freshwater. However an extension was unlikely in view of the failure of prolactin treatment in eliciting an improvement in the defective mineral balance of ablated flounders. Previous investigations have shown that the impaired osmoregulation of hypophysectomised teleosts is due primarily to the absence of endogenous prolactin secretion. Replacement therapy with the exogenous hormone promotes survival in those hypophysectomised teleosts that die in freshwater (Fundulus heteroclitus, Pickford and Phillips, 1959, Pickford et al, 1965; Poecilia latipinna, Ball and Olivereau, 1964; Gambusia sp., Chambolle, 1966; Tilapia mossambica, Dharmamba et al, 1967; Xiphophorus spp., Schreibman and Kallman, 1964,1966). This beneficial effect of the hormone is due to a complete or partial reversal of reductions in the plasma sodium (and chloride) levels in ablated fish. The main action of prolactin is to reduce the passive sodium permeability of the branchial membrane, thereby limiting the high branchial sodium efflux typical of hypophysectomised teleosts (Fundulus heteroclitus, Pickford et al, 1966a; Potts and Evans, 1966, Maetz et al, 1967a; Poecilia latipinna, Ensor and Ball, 1968, Ball and Ensor, 1969 ; Tilapia mossambica, Dharmamba et al, 1967, Dharmamba unpublished, 1969). The hormone has a similar action in hypophysectomised teleosts which are able to tolerate freshwater but which nevertheless exhibit a

hyponatremia and high sodium efflux (Carassius auratus, Donaldson et al, 1966, Lahlou and Sawyer, 1969a; Anguilla anguilla, Olivereau and Chartier-Baraduc, 1966, Maetz et al, 1967b). Recently Pickford et al (1970b) found that prolactin treatment reduced markedly the abnormally high levels of branchial Na + K-ATPase in hypophysectomised Fundulus heteroclitus and correlated this effect with the action of the hormone on sodium efflux.

In the present investigation ovine prolactin, administered in similar doses in two experiments, was found to be ineffective in repairing the main osmoregulatory defects in hypophysectomised flounders, namely a reduced plasma sodium (and hence a low osmolality) mediated by a high branchial sodium efflux. Although these results suggest that a prolactin deficiency was not responsible for the ionic imbalance of ablated flounders, other factors may explain the inactivity of the exogenous hormone evident from these brief experiments. In Anguilla spp. it has been shown that the interval between pituitary ablation and hormone treatment is of prime importance in determining the effectiveness of prolactin treatment. Butler (1967) found that prolactin did not influence the serum sodium and chloride levels of long-term hypophysectomised Anguilla rostrata, but Chan et al (1968) observed a slight increase in the serum sodium of Anguilla anguilla injected with prolactin 48 hr after the ablation. Olivereau and Chartier-Baraduc (1966) and Olivereau and Olivereau (1970) showed that, in Anguilla anguilla, prolactin therapy must be commenced simultaneously with hypophysectomy to be effective in retarding the decline in serum sodium; the ability to respond to the hormone was

reduced markedly in long-term operated eels. Those flounders in which blood parameters were determined had been hypophysectomised three weeks prior to prolactin treatment, and for fish used in the sodium flux measurements this period exceeded one month. In each case the long interval between surgery and hormone therapy could account for a loss of the ability to respond to prolactin. An endogenous prolactin is known to be present in the pituitary gland of the flounder (Chadwick, 1970) and it was clearly demonstrated with intact, SW-adapted fish that the flounder is susceptible to mammalian prolactin. Furthermore the action of the hormone in reducing branchial sodium exchange in the marine environment corresponds to the effects expected in ablated flounders maintained in freshwater - a reduction in the passive sodium permeability of the branchial membrane to limit the ion efflux and enhance plasma sodium. Further experiments, in which hypophysectomised fish receive maintenance therapy with prolactin, are necessary to discern the role of the hormone in the ionic regulation of the FW-adapted flounder.

It is possible that prolactin acts synergistically with another hypophysial hormone or some other factor whose source is influenced by hypophysectomy. The absence, or rapid and irreversible diminution, of a synergistic factor normally under maintenance control by prolactin might explain the inaction of the hormone in long-term hypophysectomised flounders. In hypophysectomised Anguilla anguilla both prolactin and cortisol are required to restore normal osmoregulation. Prolactin reverses the hyperhydration of muscle tissue and cortisol restores a normal electrolyte

balance; both hormones are more effective in combination than alone (Chan et al, 1968). The effects of ACTH and cortisol in the flounder were not investigated but these hormones are generally incapable of reversing the impaired osmoregulation of hypophysectomised teleosts maintained in freshwater with the exception of Gambusia sp. where both ACTH and prolactin are equally effective in promoting survival (Chambolle, 1966, 1967). It is unlikely that prolactin acts by directly stimulating the interrenal gland to secrete cortisol in most hypophysectomised teleosts (Ball and Olivereau, 1964; Ball and Ensor, 1967; Dharmamba et al, 1967; Chan et al, 1968; Olivereau and Olivereau, 1970) though an ACTH-like effect in Fundulus kansae is possible (Ball and Fleming unpublished, cited Ball, 1969b; Ball and Ensor, 1969).

CONCLUSIONS

In flounders maintained in sea water hypophysectomy does not alter the plasma content of the ions sodium, potassium, calcium and magnesium, but chloride is reduced slightly, an effect which is reflected in a similar reduction in serum osmolality. Pituitary ablation reduces sodium turnover; branchial Na:Na exchange diffusion is depressed, an effect which is presumed to extend to the Na:K exchange component responsible for the net branchial efflux of sodium ions. Since the renal efflux of sodium is not influenced by the ablation maintenance of a normal plasma sodium concurrent with a low branchial ion efflux is indicative of a reduced intestinal sodium uptake. These alterations in sodium exchange observed in hypophysectomised flounders are

attributable to an abolition of the adrenocorticotrophic control of interrenal activity and a resultant reduction in cortisol secretion. Hypophysectomy does not affect urine flow, nor its content of sodium, potassium and magnesium; both urine calcium and chloride are reduced, an enhanced renal conservation of chloride perhaps arising in response to the low plasma chloride of ablated fish. The flounder possessed the ability to secrete a hyperosmotic urine, which is probably under the influence of hypophysial factors, though it is doubtful whether this phenomenon is of physiological significance under natural environmental conditions.

In freshwater serum osmolality undergoes a considerable reduction in hypophysectomised flounders, marked reductions in plasma sodium and chloride being primarily responsible. The plasma content of potassium and calcium is normal but magnesium is reduced. Under the experimental regime described this osmotic imbalance impairs the freshwater survival of the normally euryhaline flounder. Sodium depletion in ablated fish is mediated by a high negative net extrarenal flux of sodium; branchial ion efflux is increased but the efficiency of branchial sodium absorption remains normal. Pituitary ablation does not affect the renal sodium efflux nor the aqueous permeability of the flounder, therefore these factors make no contribution to the sodium imbalance. It is highly probable that a hypophysial prolactin deficiency is responsible for the increase in passive branchial sodium permeability, since in sea water treatment with the exogenous hormone depressed branchial sodium exchange. The long interval between pituitary

ablation and hormone therapy may explain the inability of prolactin to reverse the impaired sodium balance on the assumption that maintenance treatment would prove effective. Urine flow and composition are normal in ablated flounders maintained in freshwater, with the exceptions of increases in urine potassium and calcium.

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