RENAL CONTROL OF RENIN SECRETION

AND SOME ACTIONS OF THE ANGIOTENSINS IN

THE KIDNEY OF THE TELEOST SALMO GAI RDNERI

by

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The renin-angiotensin system is a hormonal system found throughout the vertebrate phylogenetic scale with the exception of elasmobranchs and cyclostomes. This system has only been extensively studied in mammalian species to date. Renin secretion, in the mammals studied, is influenced by a number of factors such as renal perfusion pressure, renal tubular sodium levels, circulating catecholamines and the sympathetic nervous system. In fishes factors which influence renin secretion had not been investigated but it was known that dehydration of fishes caused a depletion of renal renin stores. This action may be due to two possible causes either a decline in blood volume with a consequent decrease in blood pressure or an increased blood osmotic pressure due to water loss. Therefore the effects of changing either blood pressure or blood osmolality (using sodium as the osmotically active particle) on renin release were investigated. Further some actions of the angiotensins on the trout kidney were investigated to determine the action of the renin-angiotensin system in combatting dehydration.

Since it is very difficult to assay renin per se, it is necessary to assay renin activity, i.e., to determine the amount of product formed from the renin-renin substrate reaction. This has been done by means of a bioassay but since the bioassay technique tends to be time consuming, a radioimmunoassay technique was developed.

A significant correlation between blood loss and plasma renin activity was established. Further work using an isolated non-filtering perfused kidney preparation showed that a decline in renal perfusion pressure caused an increase in renin release as evidenced by an increase in renin activity. This appeared to be a direct effect on the renin-secreting cells as sympathetic blocking agents had no effect on the response. Increasing renal perfusion
pressure has little or no effect on renin secretion but the end product of the system, angiotensin II, will apparently inhibit further renin secretion by a short-loop negative feedback type system.

Plasma sodium levels do not appear to affect renin secretion in the trout as hypertonic sodium perfusion of the isolated non-filtering kidney had no effect on renin release. Similarly, hypertonic sodium perfusion of the isolated filtering kidney had no apparent effect on renin release, which indicates that renal tubular sodium loads have no effect on renin secretion in this species.

Angiotensin II is known to have several diverse actions in mammalian kidneys but its action in the fish kidney was obscure. It was found that angiotensin I has little or no effect on urine flow rates but has an apparent antinatriuretic effect on the renal tubules. Angiotensin II on the other hand has both a diuretic and an antidiuretic effect, depending on which part of the renal vasculature is stimulated. In addition, the angiotensin II has an antinatriuretic effect which is of similar magnitude to that exerted by angiotensin I and this may also be a direct effect on the renal tubule. These data indicate that the angiotensins may have a role in the maintenance of blood volume and blood osmolarity in the trout. They also indicate that the angiotensin I may have been the primitive messenger of the system as this compound is not highly active in mammalian species.
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SECTION I GENERAL INTRODUCTION

1.0 THE RENIN-ANGIOTENSIN SYSTEM.

1.1 Renin in Mammals.

Renin is an enzyme found in modified smooth muscle cells in preglomerular arterioles of the kidney of mammals (De Muylde, 1945; Barajas and Latta, 1963; Hartroft et al., 1964; Bing et al., 1967). The enzyme acts on a protein precursor, angiotensinogen, found in blood plasma and splits off a decapeptide moiety called angiotensin I (Oparil et al., 1973). Another enzyme, which is simply called a converting enzyme, splits off two more amino acids from angiotensin I (AI). The resulting octapeptide, which is called angiotensin II, has various effects on both salt and water balance as well as being a potent pressor substance (Peters and Bonjour, 1971). In most mammals, angiotensin II (hereafter referred to as AII) acts on the zona glomerulosa of the adrenal cortex to stimulate aldosterone secretion (Peters and Bonjour, 1971).

There are numerous factors which influence the rate of renin secretion and the renin-angiotensinogen reaction in mammals. The earliest known factor is a decline in systemic blood pressure or, more specifically, intra-renal blood pressure. This results in an increase in renin secretion (Tobian et al., 1959; Skinner et al., 1963). Injection of vaso-dilating drugs results in an increase in plasma renin activity which is dose-dependent (Pellinger et al., 1973) and maximum afferent arteriolar dilation results in a maximum rate of renin release in dogs which is unaffected by other factors known to stimulate renin release (Eide et al., 1978). Thus it appeared that renin secretion was controlled by stretch receptors in the kidney preglomerular afferent arterioles, the location of the juxtaglomerular cells.
Varying concentrations of sodium in the renal tubular fluid can result in an effect on renin secretion and so it was postulated that the sodium concentration reaching the macula densa of the distal convoluted tubule controlled renin secretion. Results have been obtained from a number of studies to support or disprove this rather controversial hypothesis. Vander and Miller (1964) found that increasing sodium concentrations in renal perfusion fluid in intact kidneys caused a decreased renin release into the blood. The converse was demonstrated by Davis et al. (1967), i.e., a decline in perfusion sodium concentrations leads to an increase in renin secretion. Unfortunately, other workers showed that an increase in sodium concentrations causes renin release (Thurau et al., 1967). In vitro studies have shown that when renal cortical slices are incubated with a high sodium medium, renin release is inhibited in an almost linear fashion (Michelakis, 1971a). A more recent study has shown that increasing renal plasma osmolarity with either sodium, urea or dextrose results in an increase in renin release and that this increase ends within 15 seconds after the increased renal plasma osmolarity begins to decline (Young and Rostorfer, 1973). The authors postulate a direct effect of the increased osmolarity on the juxtaglomerular cells as the cause of the renin release.

Calcium was found, by Michelakis (1971a), to first increase and then decrease renin secretion. A later study by Lester and Rubin (1977) showed that extra-cellular calcium had little effect on the mechanism of renin secretion but an increased influx of the cation was needed for either synthesis or mobilization of renin. These authors proposed that intra-cellular release of calcium may be the signal which triggers renin secretion. Baumbach and Leyssac (1977), however, found that decreasing extra-cellular
calcium resulted in an increased renin secretion which was graded and reversible. A calcium ionophore had a similar but slower effect while lanthanum caused a significant depression of renin release. These authors suggested that basal renin release is a function of active, calcium-dependent cell volume regulation, i.e., swelling caused an increase in renin release. They further suggested that membrane-bound calcium has a direct effect on the cell membrane permeability to renin.

Another factor which has been investigated in recent years is some form of neural control. Vander and Luciano (1967) found that the sympathetic nervous system, which innervates the juxtaglomerular apparatus, plays a modifying role in renin secretion initiated by salt depletion. Infusion of catecholamines can result in an increase in renin secretion. Stimulation of the renal nerves has a similar effect (LaGrange et al., 1973). Earlier work had shown that blocking both the alpha and beta adrenergic receptors inhibited renin release (Winer et al., 1969) but the authors concluded that these receptors were renal in origin and not dependent on the renal nerves as transplanted human kidneys showed a normal renin response. Vandogen et al. (1973) found that the beta receptor blocking agent, d,l-propranolol, inhibited renin release during catecholamine perfusion of isolated rat kidneys but phenoxybenzamine, which blocks alpha receptors and reduces catecholamine uptake by cells had no effect on renin secretion during the perfusion. The beta receptor blocker can inhibit renin release caused by the action of vaso-dilating drugs (Pellinger et al., 1973). Later studies concluded that the vascular, tubular and sympathetic adrenergic mechanisms which govern renin release (in dogs) are capable of functioning independently (Osborn et al., 1977).
There are various factors which can influence the renin-angiotensinogen reaction once renin is released into the plasma. AII has been shown to inhibit renin release in rats, by a direct effect on the juxtaglomerular cells in a negative feedback type mechanism (Michelakis, 1971b). Since AII results in liberation of aldosterone from the adrenal cortex, it may be expected that aldosterone would also feedback to inhibit renin release. However, this is not apparent as aldosterone stimulates renin secretion. Adrenal insufficiency causes an increase in renin secretion but dexamethasone, a synthetic glucocorticoid, abolishes this increase (Reid et al., 1973). An increase in angiotensinogen (renin substrate) in blood plasma will result in an increase in plasma renin activity but this soon falls off, probably due to an effect on sodium balance or angiotensin feedback inhibition (Ménard et al., 1973).

Mammalian kidneys also contain a naturally-occurring inhibitor of the renin-angiotensinogen reaction, this inhibitor being a phospholipid. Osmond et al. (1973) have attributed this inhibition to lysophosphatidylethanolamine. In vivo inhibition of renin reactivity is caused by phosphatidylethanolamine but this compound has no effect in vitro, which suggests that it has to be converted to the other form. The in vivo conversion is rapid and has been attributed to a blood phospholipase which deacylates the phosphatidylethanolamine at the C₂ position. A later study has shown that the pressor response to renin may be inhibited by fatty acids in vivo but response to AII is unaffected (Kotchen et al., 1978). This study suggests that the fatty acids may modify the renin-renin substrate reaction in vivo as well as in vitro. In addition, Buşnag and Walaszek (1973) have synthesized two synthetic phospholipid inhibitors, one an oleyl and the other a palmityl derivative.
1.2 Renin in Non-Mammalian Vertebrates.

The preceding has been a very brief overview of some of the actions of the renin-angiotensin system and some of the factors affecting it in various mammalian species. However, the renin-angiotensin system is not unique to mammals. Both renin and angiotensin have been isolated from birds, reptiles, amphibians and some species of bony fishes (Capréol and Sutherland, 1968; Sokabe et al., 1969). It has not been found in cyclostomes or elasmobranchs (Nishimura et al., 1970).

The role of the renin-angiotensin system as well as the control mechanism is obscure in the bony fishes. Partial dehydration of Japanese eels, either by exposure to seawater or by exposure to air, results in an increase in plasma renin activity as well as a decline in kidney renin content. This observation has also been made for Tilapia mossambica (Sokabe et al., 1968; Sokabe et al., 1969; Sokabe et al., 1973). It has been suggested that the renin-angiotensin system plays a role in salt adaptation in euryhaline species but this does not explain the role of renin in stenohaline species, especially marine teleosts.

In summary, the renin-angiotensin system has been extensively studied in mammals with much less work done on this system in non-mammalian vertebrates. However, this hormonal system apparently arose in primitive bony fishes. Therefore, the subject of this study is some of the factors influencing renin secretion in fishes. More specifically, the questions asked were did the stretch receptor-induced renin secretion evolve in fishes and is there a sodium-induced renin response in these animals? In addition, some of the renal effects of the angiotensins will be examined to determine a portion of the physiological role of this system.
Section II. Introduction.

2.0 RENIN ANALYSIS.

2.1 Renin Measurement.

The renin-angiotensin system appears to have evolved in the primitive bony fishes (Nishimura et al., 1973) and is found throughout the animal kingdom with the exception of cyclostomes and elasmobranchs (Nishimura, 1970). In humans the renin-angiotensin system has been implicated in various hypertensive diseases and consequently the vast majority of work has been done on mammals with a view towards solving a purely medical problem.

One of the problems associated with work on this system is the inability of researchers to obtain a pure renin. Murakami and Inagami (1975) have published a report which shows a method for purifying hog renin but the yield is low (25%) and requires a 180,000-fold purification. Consequently, relatively large quantities of kidney material are required, but even so the purified product still shows some contamination or at least minor components are present; the function of these components is unknown.

2.1.1 Bioassay.

Since renin displays enzymatic activity, the most commonly used procedure is determination of the product, angiotensin I (AI), formed from the renin-renin substrate reaction. This determination was usually carried out by means of a bioassay, the rat vaso-pressor assay, as elucidated by Boucher et al. (1964). The major disadvantage of this form of assay is the relatively large quantities of blood plasma required for the incubation and extraction steps. In a clinical situation, however, these volumes are readily available without causing any serious injury to the subject and so this disadvantage is not apparent. But in small animal work the problem becomes
obvious. Consequently, the assay of Boucher was modified by various groups so that it could be used for assorted species. Plasma renin activity has been measured in marine teleosts, both glomerular and agglomerular species (Malvin and Vander, 1967; Mizogami et al., 1968), freshwater teleosts and holocephalins (Nishimura et al., 1973), amphibians (Sokabe et al., 1972), and cetaceans (Malvin and Vander, 1967). In addition, an alternate method for bioassay of mammalian renin has been described by Brown et al. (1969).

These modifications generally involve reducing the amount of blood plasma required for the assay. However, even with the large reduction in volumes required, the amount of blood necessary would still produce a significant reduction in blood volume for some of the species under study. This fact then circumscribed the nature of experiments which could be carried out.

2.1.2 Radioimmunoassay.

With the advent of easily obtainable radioisotopes and commercially made antibodies a new technique has come into prominence and this is the radioimmunoassay. The first radioimmunoassay procedures developed were for angiotensin II (AlI) due to the earlier availability of the octapeptide as an antigen. As a result of various problems with this technique a later development of a radioimmunoassay for AI was carried out and this is the technique that is most commonly used, again in a clinical situation.

2.1.2.1 AlI Radioimmunoassay. – Attempts to measure plasma AII by radioimmunoassay in unextracted plasma have produced somewhat erratic results as plasma proteins tend to interfere with antigen-antibody binding in an unpredictable fashion (Gocke et al., 1968). Unfortunately, extraction procedures frequently resulted in variable recoveries of AlI and concomitant concentra-
tion of salts, small proteins, and peptides that interfered with the radioimmunoassay (Oparil, 1977).

The extraction procedure can be improved by using an ultrafiltrate of plasma which gives an average recovery of AII of 98%. The extraction procedure does not totally remove all interfering substances and so the amount of ultrafiltrate used in the radioimmunoassay becomes critical (Ruiz-Maza et al., 1974).

One group of workers has reported a direct radioimmunoassay procedure for AII on unextracted plasma. This procedure obtained an excellent correlation between bioassay and radioimmunoassay (Mion et al., 1974) although the mean AII concentrations obtained in this study did not agree with those obtained in similar studies but different assay methods (Oparil and Haber, 1974). This may be due to individual variability between subjects and extraction techniques or may be due to interference by plasma protein.

Attempts to measure AII secreted in urine have been successful (Fubuchi, 1974). However, as an analytical tool this assay is far from successful since there is a poor correlation between simultaneous arterial and urinary AII concentrations, the latter depending more on renal perfusion than plasma AII concentrations (Oparil, 1977).

Thus it would appear that while an antibody for AII is readily available, the usefulness of such for a radioimmunoassay procedure is limited by the interference of various plasma components in the antigen-antibody binding phase. Also extraction procedures tend to be time consuming and not very reproducible.

2.1.2.2 AII Radioimmunoassay - A somewhat more reliable technique which has been developed is a radioimmunoassay for AII. Basically this technique involves the formation of AII from the renin-renin substrate reaction
and allowing this formed AI to incubate with a radioisotope labelled AI and an AI-antibody. The bound and unbound fractions of AI are then separated and quantified. Various groups have reported on the success of this technique and indeed have worked on comparing commercially available kits (Goldberg and Spierto, 1973; Michelakis et al., 1974; Poulsen and Jorgensen, 1974).

Controversy still exists over the use of this technique and most of this revolves around the choice of the incubation medium for the renin-renin substrate reaction. Problems exist with stabilizing such factors as pH, since CO₂ is apparently liberated during the reaction. This requires the use of concentrated buffers to avoid diluting the reaction mixture (Oparil, 1977). In addition the optimum pH of the reaction mixture appears to be in the range of 5.5 to 6.5 rather than physiological pH (Heise, 1975). The absolute amount of AI generated under these conditions appears to be two to four fold greater than those generated under physiological pH and so this pH range is the preferred range for the in vitro incubation step.

Another problem revolves around the choice of converting enzyme inhibitors. As AI is rapidly converted to AII (Boucher et al., 1974; Gagnon et al., 1974; Oakes and Stakes, 1974) this reaction must be inhibited in order to measure levels of AI. Beckerhoff et al. (1975) reported that a mixture of dimercaprol, 8-hydroxyquinoline and EDTA worked well at physiological pH while Oparil et al. (1974) preferred diisopropyl fluorophosphate with EDTA at acid pH. Poulsen and Jorgensen (1974) did not use converting enzyme inhibitors but rather added the antibody to the reaction mixture. Since the antibody had a higher affinity for AI than the converting enzyme, a peptidase, the AI bound preferentially to the antibody. Later labelled AI was added to the reaction mixture in a large volume to dilute and thus end the renin-renin
substrate reaction. The mixture was then allowed to equilibrate and the normal radioimmunoassay procedure carried out.

The variety of procedures and enzyme inhibitors tends to make comparisons of studies difficult. However by comparing the data obtained from a radioimmunoassay and from a standard bioassay, some of the difficulties may be removed.

2.1.3 Summary.

In summary, there are three possible means of measuring plasma renin activity and these are a radioimmunoassay for AII, a radioimmunoassay for AI, and a bioassay for AI. The direct measurement of circulating plasma renin levels is not practical at this time due to the absence of a readily available standard pure renin preparation and so renin activity in terms of its enzymatic function must be measured. This requires blood plasma in varying amounts and may cause experimental difficulties depending on the plasma volumes required.

2.2 Characterization of Renin.

2.2.1 Mammals.

The juxtaglomerular cells of the kidney have been shown to be the source of renin in those animals which produce this enzyme (Pitcock et al., 1959; Tobian et al., 1959; Hartroft et al., 1964). These cells appear to be modified smooth muscle cells containing granules which are homogenous, dense and osmiophilic. In addition, these granules bear a close resemblance to the beta cell granules of the pancreatic islets (Barajas and Latta, 1967). These juxtaglomerular cell granules stain specifically with Bowie's stain and weakly with aldehyde-fuchsin while periodic acid-Schiff reagent stains all granules of the juxtaglomerular cells (Krishnamurthy and Bern, 1969).

The juxtaglomerular cells are part of a certain anatomical arrangement
known as the juxtaglomerular apparatus. This consists of the afferent and efferent arterioles, the macula densa (a specialized area of the distal convoluted tubule) and a network of cells which tie the other two components together; this network being variously known as the lacis or the Polkissen or colour cushion (due to its staining properties) or the extraglomerular mesangium (Sokabe et al., 1969).

Renin has certain properties which aid to distinguish it from the peptide hormones. These properties are as follows: it is heat-labile, non-diffusible through cellophane and destroyed by acidification to pH 2.0 (Skeggs et al., 1967). Renin is also considered present in a kidney if saline extracts of that kidney produce a potent pressor substance upon incubation with homologous plasma (Chester Jones et al., 1966).

While renin is most closely associated with the kidneys, recent work has shown that renin, or at least renin-like substances, are found in many other tissues throughout the body. Renin has been located in the sub-maxillary glands of mice (Menzie et al., 1974), in various parts of the brain (Sakai et al., 1974), and is thought to originate in the chorionic membrane of the foetus (Symonds et al., 1968). In addition, Boucher et al. (1974) have postulated the existence of a new enzyme which leads to the direct formation of AI I from renin substrate. These authors call this enzyme tonin (located in the rat sub-maxillary glands) and base their hypothesis on the fact that it is not inhibited by any renin inhibitors.

2.2.2 Fishes.

Renin has been demonstrated in various species of bony fishes by a number of workers. Capréol and Sutherland (1968) carried out a comparative histological study on a number of fish species and were able to demonstrate renin granules by means of the Bowie's staining technique, in afferent
glomerular arterioles in all species tested, with the exception of salmonids and elasmobranchs.

Incubating kidney saline extracts with homologous plasma has resulted in the formation of a pressor substance in a large number of species and failure to do so in a number of others. Cyclostomes do not appear to possess any hormone resembling renin as defined by these criteria (Nishimura et al., 1970) although incubating lamprey kidney extract with purified dog substrate resulted in the formation of a pressor substance (Nishimura and Ogawa, 1973). These data result in the possibility that the lamprey may possess a renin-like substance but is lacking the substrate upon which it could act. Elasmobranchs do not have renin or renin substrate (Nishimura et al., 1970). Dogfish show a pressor response to AII but this appears to be mediated by epinephrine, as dogfish vascular beds which lack chromaffin tissue respond to epinephrine but not to AII (Opdyke and Holcome, 1978). Both freshwater and marine osteichthytes have a renin-angiotensin system (Malvin and Vander, 1967; Mizogami et al., 1968). This apparently includes salmonids despite the lack of histological evidence (Sokabe et al., 1968). Renin also has been demonstrated in lungfish and coelacanths (Nishimura and Ogawa, 1973; Blair-West et al., 1977).

Extra-renal sources of renin have not been extensively reported in teleosts. The main work in this area has been concerning the corpuscles of Stannius, glands which are generally located on the kidney of teleosts (Stannius, 1839). The cells of these glands display granules which appear similar to renin granules. However, attempts to show that these glands are part of the renin-angiotensin system have given equivocal results (Chester-Jones et al., 1966; Chester-Jones et al., 1969). Indeed, Bailey and Fenwick (1975) showed that the blood pressure effect exerted by these glands was
probably an indirect effect as a result of disturbed calcium homeostasis in which the corpuscles of Stannius are directly involved.

2.2.3 Summary.

To summarize, renin is found in the kidneys of mammals and fishes. Mammals also display many extra-renal sources of renin but this has not been extensively investigated in fishes. Renin displays certain properties: non-diffusible through cellophane, heat-labile and destroyed by acidification to pH 2.0 or lower. Also incubating saline kidney extracts with homologous plasma should result in the formation of a pressor substance.
Section II. Methods and Materials.

2.3 Localization and Characterization of Renin in Trout.

2.3.1 Experimental Animals.

Adult rainbow trout (*Salmo gairdneri*) of either sex were used throughout this study. These were obtained from the Sun Valley Trout Farm in Haney, B. C. and ranged in length from 30-45 cm and weight from 250-500 gm. The fish were maintained in dechlorinated tap water in large circular outdoor tanks. Water temperature ranged from 6° to 14°C and photoperiod was not controlled. Food consisted of Clarke's New Age High Efficiency \( \frac{1}{4} \)" fish pellets (Clarke-Moore Co. Inc., Salt Lake City, Utah).

2.3.2 Histology.

Adult rainbow trout of either sex were killed by a sharp blow to the head. A longitudinal incision was made in the mid-ventral line stretching from the vent to the transverse septum. Blocks of kidney tissue were excised and fixed for either 24 or 48 hours in Zenker-formol fixative. The fixed tissue blocks were then dehydrated in a series of alcohol baths, cleared in xylol and prepared for sectioning by infiltrating with molten Paraplast (Sherwood Medical Industries) for 24 hours. The blocks were then cut into 7 μm sections on a microtome and the sections fixed to glass slides by albumin. The sections were rehydrated and treated with Lugol's iodine solution to remove excess mercury. Following this treatment the sections were stained by the Bowie's staining technique (Bowie, 1935-1936) and covered with glass cover slips which were fixed to the slide by Permount (Fisher Scientific).

The slides were then allowed to dry and examined microscopically for the presence of renin granules.
In addition to trout, kidney blocks from several other species of fish were examined for the presence of renin granules. These fish were skilfish, *Erilepis zonifer*, pirarucu, *Arapaima gigas*, and jeju, *Hoplerythrinus unitaeniatus*.

2.3.3 Characterization of Renin.

2.3.3.1 Dialysis Effects - Plasma samples were treated as in the bioassay procedure described in Section 2.4.2 to determine if the pressor activity could be removed by dialysis. Other plasma samples were assayed by the bioassay procedure except dialysis of the plasma was not carried out to determine if the pressor activity was present before dialysis.

2.3.3.2 pH Effects - Plasma samples were acidified by adding commercial stock HCl until a pH of 2.0 or less was reached. These acidified samples were then assayed by bioassay to determine if pressor activity was still present.

2.3.3.3 Temperature Effects - Plasma samples were heated to 80°C in a water-bath for 15 minutes. These samples were then assayed by bioassay to determine if pressor activity was still present.

2.4 Assay Techniques.

2.4.1 Plasma Collection.

Blood was collected from a dorsal aorta cannula which was installed as follows. The fish was anaesthetized in a 1:20,000 aqueous solution of MS-222 (Sandoz) until it could be picked up without any evidence of struggling. A nose-cone of Intramedic PE-200 (Clay-Adams Inc.) was inserted through a hole punctured through the snout anterior to the external nares. The dorsal aorta was then punctured at the level of the first gill arch by means of a Medicut (Sherwood Medical Industries Inc.) #16 intravenous cannula. The
needle was withdrawn leaving the plastic sleeve in place and a cannula of Intramedic PE-60, which was filled with heparinized (2 I.U. heparin/ml) Cortland (Wolf, 1963) saline, was inserted into the dorsal aorta through the sleeve which was then withdrawn. The cannula was sutured to the roof of the mouth, the free end passed through the nose-cone and a length of surgical silk was tied tightly around the nose-cone where it exited from the snout. The purpose of this length of thread was to secure both the nose-cone and the cannula from sliding back and forth in the mouth.

Blood was withdrawn through the cannula as rapidly as possible and collected in a heparinized 10 cc Plastipak (Becton, Dickinson Co. Canada Ltd.) syringe and then transferred to an ice-cold polystyrene disposable culture tube (Fisher Scientific). It was then centrifuged in a refrigerated centrifuge at 1,400g and 0°C for 15 min, the plasma collected and the cells discarded.

2.4.2 Bioassay.

One milliliter of plasma was dialyzed at 2°C for 24 hours against 0.22% disodium EDTA. The dialyzed plasma was then added to 1 ml of Dowex (Dow Chemical Co.) 50W-X2 resin, NH₄⁺ form, and 0.1 ml ammonium EDTA. This reaction mixture was then incubated at 20°C for 24 hours.

Generated angiotensin was eluted from the resin by first transferring the reaction mixture to a water-jacketed chromatography column where it was washed with ammonium acetate buffer (pH 6.0), 10% acetic acid and distilled water. Elution was carried out with a 0.2 N aqueous diethylamine solution and 0.2 N ammonium hydroxide. The eluate was evaporated to dryness under vacuum and washed four times with 80% ethanol which was evaporated under vacuum. The residue was then resuspended in 0.9% aqueous NaCl solution which contained 0.001% Tween 20 (J. T. Baker Chemical Co.). To test the accuracy
of the extraction procedure known quantities of synthetic angiotensin I (Calbiochem), type Asp^1, Ileu^5, dissolved in 1 ml of Cortland saline were added to 1 ml of the resin and treated as a plasma sample. Recoveries were found to range from 90 to 104% in a series of 8 trials with 5 runs per trial. This is a modified procedure of Boucher et al. (1964).

A series of angiotensin II (Hypertensin-CIBA) solutions, 0, 10, 20, 40, 60, 80, and 100 ng/0.2 ml, was prepared in 0.9% aqueous NaCl and used as standards in the rat vasopressor bioassay. Male 250-300 gm Wistar albino rats were anaesthetized with an i.p. injection of urethan (Aldrich Chemical Co.) and the carotid artery cannulated with Intramedic PE 50 for blood pressure measurement and the jugular vein cannulated with Intramedic PE 50 for injection purposes. Blood pressure was measured with a Statham P23AC strain gauge pressure transducer and recorded on a Beckman RS Dynograph recorder. A standard angiotensin II pressor dose (1 µg/kg body weight) was administered and the preparation discarded if the mean pressure increase was less than 30 mm Hg.

2.4.3 Radioimmunoassay.

Plasma renin activity was assayed directly in this procedure, there were no extraction procedures. For the radioimmunoassay a NEN Angiotensin I Radioimmunoassay Kit (New England Nuclear Canada Ltd.) was used. In the recommended procedure, two aliquots of plasma to which had been added dimercaprol, 8-hydroxyquinoline sulphate (which act as converting enzyme and angiotensinase inhibitors) and maleate buffer, pH 6.0, are taken. One aliquot is incubated at 37°C and the other at 4°C for one hour. Following the incubation time 100 µl of each aliquot is added separately to 100 µl of an aqueous 125I-labelled angiotensin I-Tris-acetate (Sigma Chemical Co.) buffer, pH 7.4
at 20°C, solution and 500 µl of an aqueous angiotensin I antiserum-Tris-acetate buffer solution. This reaction mixture was allowed to incubate for 24 hours at 4°C after which 1 ml of an activated charcoal suspension was added, the mixture centrifuged and the supernatant saved and counted. A standard curve is run by adding known quantities of synthetic angiotensin I to the labelled angiotensin I and the antiserum, incubating at 4°C for 24 hours, adding the charcoal suspension, centrifuging and counting the supernatant. Both the standard and unknown counts were compared to the counts obtained from a known quantity of labelled angiotensin I to determine the percent angiotensin I bound to the antiserum.

This procedure was modified as the kit was designed for human plasma renin activities and repeated tests with fish plasma showed that following the recommended procedure gave highly inconsistent results. The first modification was in the incubation procedure, that of the kit being replaced by the following procedure (Nishimuro et al., 1977). One milliliter of plasma was dialyzed against 0.22% disodium EDTA at 2°C for 24 hours and then 200 µl of this dialyzed plasma were incubated with 20 µl of a combined aqueous solution of 1% neomycin sulphate (Sigma Chemical Co.) and 1% thimerosal (Sigma Chemical Co.) and 20 µl of 2 M ammonium acetate buffer (pH 6.0) and 0.1 mg of phenylmethane sulfonfyl fluoride (dissolved in ethanol - Sigma Chemical Co.) for 2 hours at 20°C in a polystyrene disposable culture tube (Fisher Scientific). A further modification was made by diluting the standards and tracer by half of what the kit recommends. The exceptions to this were the Tris-acetate buffer, the charcoal suspension and the antiserum solution which were made up to the recommended concentration.
buffer, pH 7.4 at 20°C, and 50 µl of $^{125}$I-labelled angiotensin I (New England Nuclear Canada Ltd.) were added to each tube except the first two which had 1600 µl of buffer and 50 µl of tracer only, these tubes were the total count controls. Also, tubes 3 and 4 had 500 µl buffer, 50 µl tracer and 50 µl bovine serum albumin solution. The rest of the tubes had either 50 µl of standard angiotensin I solution (NEN Canada Ltd.) or 50 µl of reaction mixture added. At this point 500 µl of antiserum (NEN Canada Ltd.) were added to all the tubes with the exception of tubes 1 through 4. All tubes were then incubated at 2°C for 24 hours to allow competitive binding between the tracer angiotensin and the generated angiotensin with the antiserum. A 1 ml volume of charcoal suspension (NEN Canada Ltd.) was added to each tube with the exception of tubes 1 and 2. The charcoaled tubes were then centrifuged for 15 minutes at 1500g in a refrigerated centrifuge at 0°C. The supernatant was collected and counted by liquid scintillation counting in an Isocap Programmable Scintillation Counter (Nuclear Chicago Ltd.). The fluor used was Riafluor (NEN Canada Ltd.). All counts were then compared to the total count controls. The accuracy of the assay was checked at frequent intervals by using plasma samples of known activities (NEN plasma renin activity standards) and the assay was found to be accurate within ±5%.

2.4.4 Determination of Optimum pH.

Dialyzed plasma samples were incubated at a series of different pH's, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. Activities were then measured by RIA. The purpose of this was to determine which pH gave optimum renin activity for fish plasma.

2.4.5 Determination of Optimum Temperature.

Dialyzed plasma samples were incubated at a series of different temperatures to determine optimum temperature for fish renin activity. These
Following the incubation procedure a numbered series of disposable polystyrene culture tubes was set up in an ice bath and 50 µl of Tris-acetate temperatures were as follows: 4°C, 10°C, 15°C, 20°C, 25°C, 35°C, and 37°C. The RIA procedure was used to measure the activities at different temperatures.

2.4.6 Activities.

All activities are expressed as plasma renin activity and the units used are ng of angiotensin II generated per milliliter of plasma per hour of incubation time for the bioassay and ng of angiotensin I generated per milliliter of plasma per hour of incubation time for the radioimmunoassay.

2.4.7 Comparison of Bioassay and Radioimmunoassay.

Pooled plasma samples were dialyzed as previously described and aliquots were taken for analysis of renin activity by bioassay and radioimmunoassay, sample standard curves of each may be found in Appendix 3.

2.5 Statistical Methods.

2.5.1 Correlations.

The bioassay and radioimmunoassay were compared by determining the coefficient of correlation (r). A probability value of less than 0.05 was regarded as significant and a probability value of less than 0.01 as highly significant.

2.5.2 Comparison of Means.

The Student's t test was used to determine if differences between mean pressor activities were significant. A probability of less than 0.05 was regarded as significant and a probability of less than 0.01 as highly significant.
Section II. Results and Discussion.

2.6 Characterization of Renin.

2.6.1 Histology.

Figure 2.1 shows a detail of the juxtaglomerular apparatus of the trout. Renin granules may be seen in the wall of the arteriole and below the blood vessel may be seen the glomerular capillary bed and the Bowman's capsule. The remaining cells in the figure are probably either interrenal, chromaffin or haematopoietic tissues which are also found in the trout kidney (Hickman and Trump, 1969).

Renin granules were also found in the kidney sections of the other species of fish examined. In the pirarucu and jeju renin granules tended to be farther away from the glomerulus than in the trout and skilfish (Figure 2.2).

No structure resembling the macula densa was observed in any of the species examined. However this is not surprising as the macula densa is apparently restricted to mammals although an intermediate type structure has been found in bird kidneys (Sokabe et al., 1969).

The granules observed in the wall of the arteriole fulfill the requirements for renin, that is to say they stain with the Bowie's stain and are located in the wall of the afferent arteriole close to the lumen of the vessel (Krishnamurthy, 1969). The granules are small and somewhat difficult to locate as they are not always found close to the glomerulus. Caprêol and Sutherland (1968) in their comparative study noted that these granules are frequently located distant from the glomerulus.

Unfortunately, while Sokabe et al. (1968) were able to identify (via bioassay) renin in salmonids, Caprêol and Sutherland in their study were
Figure 2.1. Cross section of trout kidney showing glomerulus (G), renin granules (RG) and afferent arteriole (AA). The remaining cells are probably supportive and glandular tissues which are found in the teleost kidney. X1000.
Figure 2.2 (A). Cross section of skilfish kidney showing the glomerulus (G), renin granules (R.G.) in the walls of the afferent arteriole (A.A.). X1000.

Figure 2.2 (B). Cross section of pirarucu kidney showing renin granules (R.G.), glomerulus (G), afferent arteriole (A.A.) and nephrons (N). The remaining tissue is probably either supportive or glandular. X1000.

Figure 2.2 (C). Cross section of jeju kidney showing renin granules (R.G.) in the wall of the afferent arteriole (A.A.) and sections of the nephron (N). X1000.
unable to identify renin granules in the trout kidney as they apparently did not stain with the Bowie's stain for this group. Accordingly, it was necessary to carry out further tests to confirm the histological results.

2.6.2 Chemical Characterization of Trout Renin.

Table 2.1 shows the effect of dialysis, heating, and acid-treatment of plasma on renin activity. Using isotonic saline as a control to determine the effects of volume injection on the mean pressure increase, it may be seen that the plasma extract possessed pressor activity following incubation whether or not the plasma was dialyzed. However, it should be noted that the dialyzed plasma sample apparently possessed a greater concentration of pressor activity than the undialyzed sample. Acidification of the plasma prior to incubation apparently destroyed the plasma's ability to form the pressor substance as did heating the plasma, since there was no significant increase in mean arterial pressure following injection of extracts from these treated plasmas.

Mammalian renin is known to be heat-labile, destroyed by acidification to pH 2.0 or lower and is non-dialyzable through cellophane (Skeggs et al., 1967). Eel (Anguilla anguilla) renin also has similar properties (Chester Jones et al., 1966) and so trout renin should possess these properties. Therefore, if the plasma forms a pressor substance upon incubation and if the formation of this pressor may be inhibited by the aforementioned treatments, then the conclusion which may be drawn from this evidence is that it is the action of renin which forms the pressor substance.

There are two possible reasons for the difference in activity manifested between the dialyzed and undialyzed plasma samples. Dialysis of the plasma before incubation is known to inhibit angiotensinases which break down the product angiotensin I (AI) (Nishimura et al., 1978). Therefore it is possible
Table 2.1  

Effect of Isotonic Saline and Plasma Extracts on Mean Arterial Pressure Increase  

Following Various Treatments of Plasma (a)  

<table>
<thead>
<tr>
<th>Injection</th>
<th>Dialyzed Plasma</th>
<th>Undialyzed Plasma</th>
<th>Acidified Plasma</th>
<th>Heated Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic Saline b</td>
<td>2.9 ± 0.38 mm Hg</td>
<td>1.7 ± 0.26 mm Hg</td>
<td>3.7 ± 0.41 mm Hg</td>
<td>1.89 ± 0.11 mm Hg</td>
</tr>
<tr>
<td>Plasma Extract b</td>
<td>6.5 ± 0.50 mm Hg</td>
<td>3.8 ± 0.33 mm Hg</td>
<td>3.5 ± 0.50 mm Hg</td>
<td>2.78 ± 0.83 mm Hg</td>
</tr>
</tbody>
</table>

a - Comparisons for each treatment made between isotonic saline and plasma extract.  
b - All values are means ± S.E.M. (N).  
c - P<0.01  
d - P<0.01
that in the undialyzed condition the generated AI is partially inactivated by the angiotensinases before it can adsorb on the chromatography resin thus giving a lower titer of activity. On the other hand there may be a difference in concentration of either renin or renin substrate in the plasma samples as in this series of tests differing plasma samples were used rather than aliquots from a pooled source. The most likely possibility is a combination of the two factors with the lack of dialysis being the major factor as activities from the undialyzed samples were consistently lower than those from the dialyzed samples. The test does prove that whatever is generating the pressor substance in the plasma is neither added nor removed from the plasma following dialysis, which is one of the properties of renin.

Renin, being a large protein molecule, is susceptible to denaturation by extreme pH levels (Skeggs et al., 1967). Indeed when concentrated HCl is added to the fish plasma a certain amount of coagulation is observed. Therefore the acid treatment should not only degrade the renin molecule itself but will probably act on the renin substrate as well, since the substrate is a plasma protein, in mammals an alpha globulin (Oparil et al., 1973).

The same reasoning would apply to heat treatment. Large proteins are denatured by extremes of heat and as in the acid treatment when the fish plasma was heated in the water bath coagulation was observed in the plasma sample. Therefore, one would expect little or no activity if both renin and renin substrate are destroyed by the heat treatment. The reason for the slight pressor activity, above that of the saline control level, is that some angiotensin may have been formed as the plasma heated before the large proteins denatured; angiotensins are known to be more heat stable than renin (Nishimura et al., 1973).
2.6.3 Summary.

In summary, granules are found in the walls of afferent glomerular arterioles. These granules stain with the Bowie's stain indicating that they are renin granules. Incubation of plasma produces an extractable vasoactive substance. Dialysis of plasma does not remove the source of this pressor substance but heat treatment and acid treatment of the plasma do. The conclusion to be drawn from these data is that the renin-angiotensin system exists in trout, pirarucu, jeju, carp and skilfish.

2.7 Renin Activity.

2.7.1 Temperature Optimum.

When trout plasma samples were incubated at various temperatures, range 4° to 37°, and the angiotensin measured by the radioimmunoassay, a steady increase in plasma renin activity was observed which apparently peaked at 35°C (Figure 2.3). The difference in activity between the 25° and 35° levels was not significant (0.1>P>0.05) but the difference between the activity at 35° and 37° is significant (P<0.05). Therefore the optimum temperature for trout renin is apparently 35°C. This compares with the mammalian renin temperature optimum of 37°C (Oparil, 1977), a temperature at which trout renin begins to lose a significant amount of activity. It appears unusual that trout renin would have such a temperature optimum for renin activity as these animals generally prefer much cooler temperatures in their habitat and indeed do not survive at 35°C (Fry, 1971). The most probable reason for this optimum temperature is that in the in vitro protocol other factors such as pH are kept constant by the use of various buffers which are not naturally occurring and thus those factors which may inhibit the reaction in vivo do not come into play. The fact that at 4°C trout renin displays approximately 30% of the activity at 35°C shows that in
Figure 2.3. The effect of temperature on renin activity. The y-axis is plasma renin activity in ng of AI formed per ml of plasma incubated per hour of incubation time. The x-axis is temperature in °C. Data points are the means plus or minus the standard error of the mean and represent the mean of 6 separate determinations. The activity at T = 35°C is significantly different from all other points with the exception of the activity at T = 25°C.
structure it varies from mammalian, specifically human, renin which is inhibited at this lower temperature level (Oparil, 1977). Thus the plasma blank for the analysis must be run at a lower temperature than that advised in the kit and it was found necessary to keep the plasma frozen as much as possible to prevent any development of activity.

Since 35°C is an unrealistic temperature for trout, the assays were generally run at a much lower temperature. The chosen temperature was 20°C as this was closer to the preferred habitat temperature yet still gave good activity (Figure 2.3). It was assumed that at this temperature level plasma renin activities from the *in vitro* protocol would be much closer to those found in the naturally occurring *in vivo* situation.

### 2.7.2 pH Optimum

From Figure 2.4 it may be seen that the pH optimum for trout renin is in the range of 6.0-6.5 pH units. This indicates that renin functions best in an acid medium. This is similar to mammalian renin which also functions best in slightly acidic conditions. Indeed the pH optima of the two species of renin appear the same, as mammalian renin has an optimum pH of 5.5-6.5 (Oparil, 1977). The amount of angiotensin formed at this pH in mammals is apparently two to four times that which can be generated at physiological pH (Oparil, 1977).

The reason for this pH optimum being at a lower level than physiological pH is obscure. There is a possibility that since kidney cells are known to be somewhat acidic (Koch, 1974), that blood within the renal circulation may become slightly acidic and renin evolved to function in a slightly acid environment. On the other hand arterial blood is high in oxygen and low in carbon dioxide, as compared to venous blood (Eddy, 1976) and thus tends to be slightly more basic, again when compared to venous blood. Thus renin which
Figure 2.4. The effect of pH on renin activity. The y-axis is plasma renin activity in ng of AI formed per ml of plasma incubated per hour of incubation time. The x-axis is pH in pH units. The activities at pH 6.0 and 6.5 are significantly different from all other points with the exception of the activity at pH 7.0. The points on the graph are the means ± S.E.M. of 7 determinations.
is released into the arterial side of the renal circulation would be somewhat inhibited until the venous circulation was reached. But, the bulk of published reports indicates that the primary site of action of the renin-renin substrate reaction products are the afferent and efferent glomerular arterioles. The possibility exists that the acid \textit{in vitro} medium is not affecting the renin-renin substrate reaction per se but rather is actually activating renin which has been released in an inactive form known as "prorenin", as this prorenin is known to be acid-activated (Peach, 1977). Thus, in the \textit{in vitro} system there is simply a greater concentration of active renin at acid pH than at physiological pH giving the impression that the renin-renin substrate reaction has a slightly acidic optimum pH. Also a slightly acidic pH in the \textit{in vitro} system may cause some slight deformation of the structure of the renin substrate making the leucine-leucine bond that the renin cleaves more available. However, this is still in the realm of speculation as studies on prorenin and pH of renal blood are still in the preliminary stage and consequently there are not much data on this subject.

2.7.3 Comparison of Assay Techniques.

Initial experiments showed that trout Al apparently bound to human Al antiserum and so it appeared possible to use a radioimmunoassay technique for measuring plasma renin activity. However it was necessary to compare the data obtained for renin activity from the radioimmunoassay (RIA) with activities which were obtained via bioassay (BA) to ensure that what was binding to the antibody was in fact trout Al and that it was competing equally with the labelled exogenous Al. The results of this study may be seen in Figure 2.5. From this figure it may be observed that there is a good correlation between plasma renin activity (PRA) obtained via RIA and BA. The correlation coefficient of 0.93 is significant (P<0.05). However the
Figure 2.5. Correlation between activities obtained from RIA and BA. The y-axis is plasma renin activity obtained from the radioimmunoassay and is measured in ng AI formed per ml of plasma incubated per hour of incubation time. The x-axis is plasma renin activity obtained from the bioassay and is measured in ng AII per ml of plasma per hour. The line equation is $y = 0.09x + 0.04$ and the correlation coefficient of 0.93 is significant at the 0.05 level.
fact that the BA gave PRA's which are approximately ten fold greater than those PRA's obtained as a result of RIA suggests that the trout Al is only partially bound to the human Al antiserum. This is not surprising as Nakajima et al. (1971) found that teleost angiotensins appear much more basic than the mammalian forms. But since the correlation between the two is quite good, RIA was used as the method of choice for determining PRA's. While the results using the NEN antibody are quite good, those obtained from using the Squibb antibody were better, giving a correlation coefficient of 0.984 (Nishimura, pers. comm.). But the NEN method was used as it gave good results and was readily available.

The reasons for using a RIA technique over a BA technique are many and varied. Primarily the RIA technique is faster in terms of actual time spent on the incubation and extraction procedures and less expensive in terms of number of animals required. That is the BA technique requires both trout and rats, the trout being the experimental animals and the rats being used to measure the amount of angiotensin formed. In addition there is a certain amount of variability between the animals in the BA which can complicate interpretation of results. It is true that this variability can be overcome in a large part by artificially depressing the rat's blood pressure so as to render the assay more sensitive but this procedure also adds to the complexity of the BA. One other problem may occur during the extraction procedure as recovery from the chromatography resin of the generated angiotensin may vary from 90-104%, although in this study the recovery of activity from the resin averaged 95%, using either synthetic Al or AII as controls.

One main advantage of the RIA over the BA is that the RIA involves only one enzymatic step, i.e., the generation of Al, while the BA involves two.
AI is generated during the incubation period, absorbed on the chromatography resin and extracted and redissolved in isotonic saline. It is this solution which is injected into the rat and there is a lag phase between time of injection and increase in mean blood pressure which indicates that the AI must be converted to AII. It is entirely possible that some of the activity may be lost by alternate metabolic routes during the conversion as these are known to exist (Freeman et al., 1978) or by binding of the rat-generated AII to some other receptor than the vascular smooth muscle receptor. The RIA which is carried out in an entirely in vitro situation would not face these problems.

Problems with the RIA system do exist however. When using an antibody made for human AI to bind trout AI there is the definite possibility that the labelled exogenous AI may out-compete the trout-generated AI for binding to the antibody. Indeed this has happened when the RIA is used to measure eel (Anguilla sp.) PRA, i.e., eel AI will not bind to the antibody (Nishimura et al., 1978). In addition it has been observed by other workers that the choice of converting enzyme inhibitor must be based on more than the pH optimum of the inhibitor; in addition the effect of the inhibitor on the renin-renin substrate reaction must be taken into account as some inhibitors will adversely affect the renin reaction (Nishimura, pers. comm.). On the other hand, the technique is generally quick and easy to carry out, which are definite facts in its favour.

2.7.4 Summary.

In summary, trout renin has its temperature optimum, in vitro, of 35°C and a pH optimum, again in vitro, of 6.0-6.5 pH units. Trout plasma renin activity may be measured by radioimmunoassay using a commercially available
kit as trout AI is similar to human AI in its binding characteristics to an antibody which is made specifically for human AI.
Section III Introduction.

3.0 CONTROL OF RENIN SECRETION: PRESSURE EFFECT.

3.1 Intrarenal Control of Renin Secretion.

3.1.1 Renal Ischemia.

It has long been known that certain kidney diseases result in chronic sustained hypertension. Attempts to explain this phenomenon were not very successful until the work of Goldblatt et al. in 1934. This group devised a technique whereby a variable clamp was placed on either one or both of the renal arteries to render the kidney(s) ischemic. They found that when the renal arteries were partially occluded, there was a sustained increase in systemic blood pressure and when the clamps were removed the systemic blood pressure would soon return to normal (pre-operative) levels. As a result of this type of study it was initially postulated that the hypertension was a result of renal ischemia, possibly due to anoxemia and/or hypercapnia caused by this ischemia. Then, in 1942, Huidobro and Braun-Menendez examined the effect on renin output in dogs when the animals were breathing 7-8% oxygen and 5% carbon dioxide and unfortunately for this hypothesis did not find a significant change in renin secretion. These authors concluded that renal anoxemia and hypercapnia were not major controlling factors in renin release. Reduction of arterial oxygen levels, from 96% to 56%, had no discernible effect on renin secretion (Skinner et al., 1963; 1964a). As a result of these studies the hypothesis that renal ischemia controlled renin secretion was partially discredited.

3.1.2 Pulse Pressure Hypothesis.

Concurrent with the ischemia studies other groups were examining the possibility of a haemodynamic signal for renin release. When isolated dog
kidneys were perfused, an increase in renin output was observed, this in association with a decreased pulse pressure (Kohlstaedt and Page, 1941). It was then concluded that decreased renal blood flow was a consequence of increased renin production due to a decrease in pulse pressure. However, later work showed that renal artery stenosis resulted in an increase in renin release, regardless of pulsatile or non-pulsatile flow (Kolff, 1958).

In 1964-1965, Skinner et al. examined the pulse pressure hypothesis during their studies on renal ischemia. They found that compression of the kidney, by means of an oncometer, resulted in an increased renin release without any significant change in renal blood flow. Also constriction of the renal vein so that renal blood flow was decreased by 50% had no effect on renin secretion although an increased renin output occurred during suprarenal aortic constriction even when the reduction in renal perfusion pressure did not produce a change in renal blood flow. In addition, increased renin output is observed in dogs with high-output heart failure as a result of a large arteriovenous fistula (Davis et al., 1964). All these studies argued against a decreased pulse pressure being the cause of renin release but rather some other factor, such as mean renal perfusion pressure, especially since in the latter study the animals displayed a marked widening of the pulse pressure.

### 3.1.3 Renal Perfusion Pressure Hypothesis.

Tobian et al. (1959) observed decreased granulation in the juxtaglomerular (JG) cells with a rise in renal perfusion pressure (in the isolated rat kidney) and they suggested that the JG cells may act as stretch receptors and thus change their rate of renin secretion as the arteriolar wall changes its degree of stretch. But as these authors pointed out, changes in renal blood flow and/or glomerular filtration rate were probably
associated with this increased perfusion pressure, so there was a possibility that the increased renin secretion may have been a result of these factors as well. The work of Skinner et al. (1963; 1964a) supported the perfusion pressure hypothesis but this work has been criticized for the authors' failure to control such factors as changes in renal tubular sodium and glomerular filtration rate which could possibly have an effect on the rate of renin secretion (Blaine et al., 1970; Davis et al., 1971; Davis and Freeman, 1976).

As a result of the realization of the limitations of this earlier work a new approach was developed, that of the non-filtering kidney model (Blaine et al., 1970). This model thus prevented any tubular effects on renin secretion or so it was assumed as the macula densa was rendered non-functional. Experiments were done to determine the effect of haemorrhage and aortic constriction (Blaine and Davis, 1971; Blaine et al., 1971); plasma renin activity was observed to increase markedly after these experimental manoeuvres. These authors then concluded that there was definitely an intrarenal vascular receptor which controlled renin release.

The non-filtering kidney model was later improved so as to cancel all possible neural and humoral factors. This was done by denervation of the kidney and adrenalectomy to exclude adrenal steroids and catecholamines (Blaine et al., 1971; Davis et al., 1971). These authors then found that the renin response to haemorrhage or aortic constriction was not abolished but rather more marked than in the simple non-filtering model. Therefore, by the isolation of the JG cells and renal afferent arterioles from the influence of the macula densa, the renal sympathetic nerves and the catecholamines, the presence of a renal vascular receptor which responds to changes in renal perfusion pressure is convincingly demonstrated.
Additional studies have provided further evidence to support the renal vascular receptor hypothesis. Isolated dog kidneys respond to ureteral clamping by increased renin release while a subsequent increase in renal arterial pressure decreased renin release to the control level. Conversely, an initial increase in renal perfusion pressure decreased renin which was returned to the control level by ureteral clamping (Kaloyanides et al., 1973). High perfusion pressures in isolated rat kidneys have depressed renin release while low perfusion pressures have stimulated renin release in this preparation (Hofbauer et al., 1974). Churchill et al. (1974) found an inverse relationship between changes in renal perfusion pressure and plasma renin activity when aortic clamping is superimposed on ureteral occlusion, which also supports the renal vascular receptor theory.

Having determined that a renal vascular receptor exists, the next problem is to determine the location of this receptor, i.e., in what part of the vascular tree it is located. The studies designed to ascertain this location used papaverine which is known to block renal autoregulation, an afferent arteriolar function (Davis et al., 1972). These authors infused papaverine into the renal artery of a denervated non-filtering kidney, then haemorrhaged the experimental animal in the amount of 20 ml/kg and found that the renin response to haemorrhage was completely blocked. However, in denervated filtering kidneys the renin response to haemorrhage still persists despite infusion of papaverine. Davis et al. (1972) then concluded that the data supported the hypothesis of the renal vascular receptor and that this vascular receptor was probably located in the afferent arterioles since papaverine is known to dilate the renal afferent arterioles as it prevents renal autoregulation. Furthermore in dogs with a thoracic vena caval constriction and either a filtering or non-filtering kidney, papaverine
produced a striking fall in renin secretion, an increase in renal blood flow and a decrease in renal vascular resistance (Witty et al., 1971). In sodium-depleted dogs with a denervated kidney, papaverine infusion caused an 85% fall in renin secretion and a decrease in renal resistance while papaverine had no discernible effect in normal control animals (Gotshall et al., 1974). All of these observations point towards an afferent arteriole locus for the renal vascular receptor.

Corsini and Bailie (1973) (reported in Davis and Freeman, 1976) found that furosemide increased renin secretion in adrenalectomized dogs with a denervated non-filtering kidney, in association with an increase in renal vascular resistance. Papaverine alone produced a maximal decrease in renal resistance as renin output doubled. But furosemide did not have any effect above that produced by papaverine on the renal arterioles and renin secretion did not increase further. These observations are consistent with the hypothesis that renin response is mediated by a renal vascular receptor which responds to decreases in renal perfusion pressure. This hypothesis is also known as the baroreceptor response hypothesis.

3.1.4 The C.N.S. and the Renal Vascular Receptor.

Having established that there is a renal vascular receptor in the afferent arterioles which causes renin release in response to decreased renal perfusion pressure the next question to be resolved concerns the role of the sympathetic nervous system in this response. The kidney appears well innervated by sympathetic fibres (Ham, 1965; Barajas and Latta, 1967) and thus there exists the possibility that the vascular receptor is under neural control.

Direct stimulation of the renal nerves results in an increase in renin secretion (Vander, 1965; Loeffler et al., 1972). However, since in those
studies the stimulating electrode was placed around the renal artery and nerves, there existed the possibility that the partial occlusion of the artery was causing the response rather than the stimulation. Therefore, Johnson et al. (1971) dissected the nerve free from the artery and stimulated the freed nerve. They found that renin secretion increased as a result of this stimulation. In addition, circulating catecholamines have been shown to cause renin release in denervated kidneys (Johnson et al., 1971).

Buñag et al. (1966) showed that the acute response in renin release to haemorrhage was not affected by blocking ganglia or by using a local anaesthetic on the renal nerves. Another group (Hodge et al., 1966) found that renin response to non-hypotensive haemorrhage was blocked by blocking the renal nerves. This seemed to indicate the presence of a neural-hormonal link. Weber et al. (1974) have suggested that renin response to a non-hypotensive haemorrhage is a result of sympathetic activity rather than an action of the renal vascular receptor. Johns and Singer (1974) found that propranolol would only block renin release if this release was caused by adrenergic stimulation. In another study, methoxamine, an alpha adrenergic stimulator, could not be shown to stimulate renin release (Vandogen and Peart, 1974).

In a more recent study, Osborn et al. (1977) found that propranolol would only partially block furosemide-induced renin release in the denervated non-filtering kidney preparation. These authors concluded that the vascular response and sympathetic response were capable of acting independently. Davis and Freeman (1976) in their review concluded that the acute renin response to haemorrhage is mediated only in part by the sympathetic nervous system, especially as a denervated kidney is capable of responding to a decrease in renal perfusion pressure.
3.1.5 Summary.

In summary, renin secretion from the kidney appears to be mediated by an intrarenal vascular receptor. This receptor appears to be located in the afferent arteriole and does not require innervation in order to function. It responds to a decrease in renal perfusion pressure rather than ischemia or a change in pulse pressure.

3.2 Vascular Control of Renin in Fishes.

There is little published information available on the vascular control of renin release in fishes. The only indication that this is a possibility is that dehydration of either Japanese eels or *Tilapia mossambica* causes an increase in plasma renin activity and a decrease in juxtaglomerular cell renin content (Sokabe et al., 1966; Sokabe et al., 1968; Sokabe et al., 1973). This could be a result of a decline in renal perfusion pressure due to water loss and a consequent fall in blood volume. Therefore the question was asked, is there a renal vascular receptor which controls renin release in fishes?
Section III Materials and Methods.

3.3 Kidney Perfusion Preparation.

The animals used in this study were adult rainbow trout obtained and maintained as previously described (see Section 2.3.1). The fish were anaesthetized in a 1:20,000 aqueous solution of MS-222 (Sandoz) and the dorsal aorta cannulated as previously described (Section 2.4.1). The caudal peduncle was then severed and a cannula of Intramedic PE 60, filled with heparinized saline (10 I.U. heparin per ml), was inserted into the caudal artery and pushed anteriorly as far as the vent. The cannula was then secured in place by means of silk sutures which encompassed the artery and the vertebral column.

A longitudinal incision was made in the mid-ventral line of the animal which stretched from the vent to the posterior border of the heart. The ureters were then located and tied off by means of silk sutures. The viscera including the swim bladder were then freed from the body wall by blunt dissection and the blood vessels leading to the swim bladder were tied off and cut, the purpose of this being to prevent leakage from these severed vessels. The viscera were then removed and the blood vessels leading to the viscera from the aorta were tied again to prevent leakage. The removal of the viscera exposed several segmental vessels in the body wall; these vessels were then cauterized by hot-wire cautery.

The pericardial cavity was then opened and the heart exposed. An Intramedic PE 90 cannula was then implanted in the ventricle via the ventral aorta and bulbus arteriosus and tied into place by means of silk sutures. During the entire surgical preparation the animal's gills were kept irrigated with a 1:200,000 chilled, aerated, aqueous solution of MS-222.
The completed preparation was transferred to a bath of Cortland saline which was placed in an ice-bath so that the preparation could be kept cold. The dorsal aorta cannula was used as a manometer to measure perfusion pressure, the caudal artery cannula was used as the perfusion cannula and the heart cannula as the collecting cannula as is diagrammatically represented in Figure 3.1.

The preparation was then perfused with aerated Cortland saline under constant pressure and flow for at least 60 minutes. This preliminary perfusion period was the clearing period, that is all the blood remaining in the preparation would be replaced by perfusate. Also, if during this time the perfusion pressure, as measured by the dorsal aorta cannula, fell below 15 cm H$_2$O the preparation was discarded. During the experimental period, perfusion pressure could be altered by changing the height of the perfusion bottle and reservoir (see Figure 3.1).

3.4 Experimental Procedures.

3.4.1 Effect of Blood Withdrawal.

Varied amounts of blood were withdrawn from intact animals as described previously (Section 2.4.1). The blood was then centrifuged and the plasma assayed for renin activity by bioassay as previously described. The fish were anaesthetized and weighed to establish a ground for comparison of plasma renin activities. The data were then examined to determine if blood loss and plasma renin activity were related.

3.4.2 Effect of Perfusion Pressure.

A kidney perfusion preparation was set up as described in Section 3.3 and the kidney perfused for 90 minutes, following the clearance period, at a pressure head of 35 cm H$_2$O. Outflow perfusate samples were collected at 0, 30, 60, and 90 minutes in disposable polystyrene culture tubes (Fisher
Figure 3.1. Diagrammatic view of the kidney perfusion preparation and apparatus. The trout carcass was held in the upright position by a small piece of sponge wedged between the fish and the wall of the bath. In addition, the collecting cannula led over the side of the bath and not the end as it appears in the diagram.
Scientific); the tubes were sealed with Parafilm (American Can Co.), frozen and stored for later assay.

At this time the perfusion pressure head was lowered 10 cm and the kidney perfused at the lower perfusion pressure for a further 90 minutes. Outflow perfusate samples of 2 ml each, same volume as the earlier samples, were collected at 100, 130, 160, and 190 minutes in disposable tubes, the tubes sealed with Parafilm, frozen and stored frozen for later assay.

Perfusate renin activity was measured by adding 0.5 ml of perfusate to 0.5 ml of pooled plasma stock and mixing thoroughly. This mixture was then treated with the radioimmunoassay procedure. Control samples were simply 0.5 ml of Cortland saline and 0.5 ml of the plasma stock.

3.4.3 Alpha Receptor Blockers.

The alpha adrenergic blocking agent, phenoxybenzamine (Smith Kline & French I.A.C.), was added to the perfusate and the experimental procedure outlined in the previous section carried out.

3.4.4 Beta Receptor Blockers.

The beta adrenergic blocking agent propranolol (Ayerst, McKenna & Harrison Ltd.) was added to the perfusate and as before, the experimental procedure previously described carried out.

3.4.5 Combined Receptor Blockage.

In this series of experiments both propranolol and phenoxybenzamine were added to the perfusion solution. The kidney was then perfused as in the earlier description.

3.4.6 Increasing Perfusion Pressure.

In this series of experiments the kidney perfusion preparation was used. The preparation was allowed to clear and stabilize for 60 minutes at the 35 cm perfusion head. The perfusion head was then lowered 10 cm and the kidney
perfused for 90 minutes. The time when the perfusion head was lowered was regarded as zero time. Samples were taken at times 0, 30, 60, and 90 minutes.

At this time the perfusion head was raised 10 cm and the kidneys perfused for a further 90 minutes. Samples were taken at times 100, 130, 160, and 190 minutes. The perfusate renin activity was assayed as described in Section 3.4.2.

3.4.7 Angiotensin II and Renin Release.

Adult trout of either sex were killed by a blow to the head, a mid-ventral incision was made in the body wall and the kidney quickly excised. The excised kidney was placed in ice-cold Cortland saline and the posterior third portion divided into six equivalent blocks. One piece of tissue was then placed in each of six petri dishes containing 2.0 ml aerated Cortland saline and 0, 50, 100, 150, 200, and 250 ng of angiotensin II (Hypertensin - CIBA) respectively. The tissue blocks were allowed to incubate for 15 minutes and a 0.5 ml sample of fluid was taken from each petri dish. These samples were assayed for renin activity as previously described.

3.4.8 Perfusion Solutions.

Perfusates consisted of Cortland saline with 4% polyvinylpyrrolidinone (PVP), average molecular weight - 40,000 (Matheson, Coleman and Bell Manufacturing Chemists), added to simulate the oncotic pressure of the plasma proteins.

In the alpha blocking experiments phenoxybenzamine was added to make a final concentration of \(2 \times 10^{-5}\) grams per milliliter perfusate. In the beta blocking experiments propranolol was added to the perfusate to make a final concentration of \(10^{-6}\) grams per milliliter perfusate. Identical concentrations were used in the combined blocking experiments, i.e., \(2 \times 10^{-5}\) g phenoxy-
benzamine and $10^{-6}$ g propranolol per ml of perfusate.

In the *in vitro* experiments Cortland saline with 4% PVP added was used as the incubation medium.

In all cases the perfusate or incubation medium was first filtered through Whatman No. 1 (W. & R Balston Ltd.) filter paper and then through a Millipore filter, type HA and pore size 0.45 μM (Millipore Corporation) to remove any particulate matter which could clog capillaries and thus increase resistance to flow.

3.4.9 Statistical Methods.

Linear regression analysis was carried out to determine the correlation coefficient ($r$) and the Student's $t$ test was used in comparison of means to determine significant differences. A P value of less than 0.05 was taken as significant and a P value of less than 0.01 was taken to be highly significant.
Section III Results and Discussion.

3.5 Effect of Haemorrhage and Changes in Renal Perfusion Pressure.

3.5.1 Haemorrhage.

There is a significant (P<0.05) positive correlation between amount of blood lost and plasma renin activity in the trout as may be seen in Figure 3.2. While it is true that this does not necessarily define a cause-effect relationship, the correlation does indicate that haemorrhage or a decrease in blood volume results in an increase in plasma renin activity. Because the blood was withdrawn as rapidly as possible via the cannula, the plasma renin activities observed in this study may be somewhat exaggerated. Rapid blood withdrawal is necessary, however, so as to minimize time lost before the plasma is frozen. It will be recalled that the fish renin-renin substrate reaction proceeds fairly rapidly even at 4°C (Section 2.7.1) and thus it is desirable to minimize the amount of time in order to minimize the reaction before assay. This response has been observed in other species. Churchill (1973) found that moderate to severe haemorrhage caused a proportionate increase in plasma renin activity in rats. This response may also be observed in rabbits (Weber et al., 1974) but minor haemorrhage, less than 10% loss of blood volume, had no effect on plasma renin activity in man (Goetz et al., 1974).

Two possible reasons may be found for the renin response to haemorrhage. Since renin is produced in the kidney vascular bed of fishes (Capréol and Sutherland, 1968; Nolly and Fasciolo, 1972) there is a distinct possibility that an intrarenal vascular receptor, such as may be found in mammals, could exist. The other possibility is that there may be a systemic receptor which influences renin release via some sort of neural pathway. Accordingly, before
Figure 3.2. Correlation between haemorrhage and plasma renin activity. The y-axis is plasma renin activity in ng AII/ml/hr while the x-axis is blood loss in terms of ml blood loss per kg body weight. For the line equation $y = 0.89x + 0.17$ the correlation coefficient $r = 0.81$ is significant at the 5% level. The number of animals used in this experiment was 15 and plasma renin activity was measured by bioassay.
$y = 0.89x + 0.17$
any conclusions may be drawn from the effects of haemorrhage on renin, it is necessary to examine the role of renal perfusion pressure in renin release.

3.5.2 Changes in Renal Perfusion Pressure.

When renal perfusion pressure is decreased by approximately 50% renin release, as measured by renin activity, shows a 60% increase. Figure 3.3 shows the overall pattern of renin secretion during the entire perfusion period. As may be seen during the first 90 minutes of perfusion (at high pressure) the renin activity shows a slight non-significant increase followed by a small decline. When the perfusion pressure is lowered (at 90 minutes) the renin activity immediately increases to a point greater than that which it had reached up to this time. Renin activity shows a steady increase from 100 min to 190 min and this increased activity is significantly higher than the activities at 0 min, 90 min, and 100 min. This shows that the renin secretion from the kidney has significantly increased. In Table 3.1 may be found the average values for renin activity during this period. Renin activity increases from 0.36 ng AI/ml/hr to 0.58 ng AI/ml/hr, an increase of 60%, while the perfusion pressure decreases from 21.7 cm H₂O to 13.1 cm H₂O, a 40% decrease. Indeed there is a highly significant correlation between renin activity and renal perfusion pressure as may be seen in Figure 3.4.

Increasing renal perfusion pressure from a low level to a high level apparently had no effect on renin activity as may be seen in Figure 3.5 and Table 3.1. In this case the initial perfusion period is done at low pressure and seems to result in an apparent decline in renin activity. However this decline is not significant. When the perfusion pressure is increased at 90 minutes the renin activity does not either increase or decrease but remains at approximately the same level as that found at 90 minutes. In Table 3.1 it may be seen that when perfusion pressure increases from 12.9 cm H₂O to
Figure 3.3. The effect of a decline in renal perfusion pressure on renin release from the isolated perfused trout kidney. The y-axis represents renin release as measured in perfusate renin activity which is in ng AI/ml/hr while the x-axis represents time of perfusion as measured in minutes. The break in the line between 90 and 100 minutes shows the point at which the perfusion pressure was changed from a high (35 cm H$_2$O) to a low (25 cm H$_2$O). Each point on the figure is the mean ± S.E.M. of 10 experimental animals.
TABLE 3.1

Effect of Changes in Renal Perfusion Pressure on Renin Release\(^a\)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Perfusion Period(^b)</th>
<th>Perfusion Pressure (cm H(_2)O)</th>
<th>Renin Activity (ng AI/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High to Low Pressure</td>
<td>Initial</td>
<td>21.7 ± 0.38(^c)</td>
<td>0.36 ± 0.07(^d)</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td></td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>13.1 ± 0.71(^c)</td>
<td>0.58 ± 0.07(^d)</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td></td>
<td>(10)</td>
</tr>
<tr>
<td>Low to High Pressure</td>
<td>Initial</td>
<td>12.9 ± 0.77(^e)</td>
<td>0.68 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td></td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>19.6 ± 0.72(^e)</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>(14)</td>
<td></td>
<td>(14)</td>
</tr>
</tbody>
</table>

\(^a\) All values are means ± S.E.M. (N)

\(^b\) Initial refers to the first 90 minutes of perfusion while second refers to the remaining 90 minutes of perfusion

\(^c\) P<0.01

\(^d\) P<0.05

\(^e\) P<0.01
Figure 3.4. The correlation between renal perfusion pressure and renin secretion in the isolated perfused trout kidney. The y-axis represents renin release as measured by perfusate (plasma) renin activity and is in ng AI/ml/hr. The x-axis is renal perfusion pressure as measured in cm H$_2$O. For the line equation $y = -11.77x + 23.93$ the correlation coefficient $r = -0.92$ is significant at the 1% level.
PERFUSION PRESSURE (cm H$_2$O)

$y = -11.77x + 23.93$
Figure 3.5. Changing renal perfusion pressure and renin release in the isolated perfused trout kidney. The y-axis represents the renin release from the kidney and is measured in perfusate renin activity, i.e., ng AI/ml/hr. The x-axis represents the time of perfusion in minutes. The break in the graph between 90 minutes and 100 minutes is the point at which the perfusion pressure was changed from the low (25 cm H₂O) to the high (35 cm H₂O). Each point on the graph represents the mean ± S.E.M. of 14 determinations.
19.6 cm H$_2$O, a highly significant increase, renin activity shows only a slight non-significant decline from 0.68 ng AI/ml/hr to 0.58 ng AI/ml/hr.

These data show that renal perfusion pressure plays an important role in renin release in the trout. However it is only a decline in the perfusion pressure which stimulates renin release as increasing the perfusion pressure has no effect on the rate of renin release. Therefore, it appears that once renin release has been initiated, the juxtaglomerular (JG) cells will continue to secrete renin until inhibited by some, as yet unknown, signal.

This response is very similar to the baroreceptor response found in mammals (Liard et al., 1974). The nature of this response was elucidated by Churchill et al. (1974) when they combined salt-loading which would inhibit renin release and a decline in renal perfusion pressure to show that the baroreceptor response overrode the sodium response. On the other hand, other groups have recently shown that the baroreceptor response is not necessarily limited to the intrarenal receptor as carotid sinus hypotension causes renin release (Jarecki et al., 1978). Also reductions in right atrial pressure can cause an increase in renin secretion (Brosnihen and Bravo, 1978).

It would appear that in the trout the receptor is an intrarenal one, since in this preparation the only vascular circuitry left substantially intact is the renal circulation and to some extent the cranial circulation. The receptor is pressure-sensitive as the changes in renal perfusion pressure were not accompanied by a change in the perfusate outflow. This shows that the flow rate through the renal vascular bed was constant. The most probable reason for the constant perfusate flow through the renal vascular bed is the presence of an intact dorsal and lateral segmental circulation. These vessels could not be cauterized as they exit directly from the dorsal aorta into the muscle mass. It thus appeared that these vessels acted as a variable resistor.
in the perfusion circuit. Therefore the change in renin secretion would not be due to a change in perfusate flow rates.

The next question which has to be asked is the nature of the receptor. These experimental data show that the signal received by the receptor is a decline in renal perfusion pressure. However, how is this signal acted on, i.e., is it a direct effect on the JG cells, or is it mediated by some sort of neural reflex mechanism? The latter is a possibility, for the kidney is well innervated by sympathetic fibres in both mammalian (Barajas, 1964; Barajas and Latta, 1967) and piscean species (Sokabe and Ogawa, 1974). In order to examine this possibility sympathetic receptor blocking studies were carried out while altering the renal perfusion pressure.

3.5.3 Blocking Studies.

Figure 3.6 and Table 3.2 show the effects of the alpha receptor blocking agent, phenoxybenzamine, on renin secretion induced by the baroreceptor response. During the period of high pressure perfusion the renin activity shows a slight, non-significant increase but when the perfusion pressure decreases the renin activity shows a massive increase until the final level is approximately 10 fold higher than the initial level. Table 3.2 shows that when the average perfusion pressure declines from 21.3 cm H$_2$O to 14.7 cm H$_2$O, a significant (P<0.05) decrease, then the average renin activity increases five fold, from 0.02 ng AI/ml/hr to 0.10 ng AI/ml/hr.

These data would indicate that the baroreceptor response of renin secretion is not controlled or effected through the sympathetic alpha receptors, since earlier work has shown that phenoxybenzamine is an effective alpha receptor blocking agent in salmonids (Randall and Stevens, 1967).
Figure 3.6. Effect of addition of the alpha blocking agent phenoxybenzamine on the baroreceptor response in the isolated perfused trout kidney. The y-axis is renin activity in ng AI/ml/hr while the x-axis is time of perfusion in minutes. The break in the line represents the point at which the perfusion pressure was changed from the high to the low. Each point on the line represents the mean ± S.E.M. of 10 determinations. The dose administered was $2 \times 10^{-5}$ gm phenoxybenzamine per millilitre of perfusate.
### TABLE 3.2

Effect of Alpha Receptor Blockage on the Renin Response to Hypotension

<table>
<thead>
<tr>
<th>Perfusion Period</th>
<th>Perfusion Pressure (cm H₂O)</th>
<th>Renin Activity (ng AI/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>21.3 ± 1.48&lt;sup&gt;c&lt;/sup&gt; (10)</td>
<td>0.02 ± 0.013&lt;sup&gt;d&lt;/sup&gt; (10)</td>
</tr>
<tr>
<td>Second</td>
<td>14.7 ± 1.33&lt;sup&gt;c&lt;/sup&gt; (10)</td>
<td>0.10 ± 0.04&lt;sup&gt;d&lt;/sup&gt; (10)</td>
</tr>
</tbody>
</table>

- All values are means ± S.E.M. (N)
- Initial refers to the first 90 minute perfusion period while second refers to the second 90 minute perfusion period.
- P<0.01
- P<0.05
In mammals, stimulation of the renal alpha receptors generally results in an inhibition of renin secretion (Vandogen and Peart, 1974). This may not occur in fishes as blocking the alpha receptors has no effect on renin release, and if these receptors inhibit renin secretion, blockage should result in an increased renin secretion during the control period.

Figure 3.7 and Table 3.3 show the effects of beta receptor blockage on hypotension elicited renin release and as may be seen there is little or no effect. Renin activity declined slightly then rose slightly during the period of high pressure perfusion. As before, the decline in the perfusion pressure produced a significant increase in renin activity as is seen in Figure 3.7. The average perfusion pressure decreased from 19.2 cm H₂O to 14.2 cm H₂O, a 26% decrease, and renin activity increased from 1.61 ng AI/ml/hr to 3.35 ng AI/ml/hr, a 100% increase (Table 3.3).

These data indicate that the beta receptors do not play a major role in hypotension induced renin secretion as blocking these receptors has no effect on the renin response. These receptors have been implicated in control of renin secretion in mammalian species. Pettinger et al. (1973) found that blocking beta receptors inhibits the increase in plasma renin activity caused by the vasodilating drugs, minoxidil and hydralazine. Renin secretion is increased when the isolated rat kidney is perfused with catecholamines but this increase is inhibited by propranolol (Vandogen et al., 1973). Intramuscular injection of propranolol, in rabbits, will cause a decrease in
Figure 3.7. The effect of addition of the beta blocking agent propranolol and changing renal perfusion pressure on renin release in the isolated perfused trout kidney. The y-axis represents renin release measured in terms of perfusate renin activity as expressed in ng AI/ml/hr. The x-axis represents time of perfusion and is measured in minutes. The break in the line between 90 and 100 minutes represents the point at which the perfusion pressure was changed from high to low. Each point on the line represents the mean ± S.E.M. of 9 determinations. The dosage administered was $10^{-6}$ gm propranolol per milliliter perfusate.
### TABLE 3.3

Effect of Beta Receptor Blockage on the Renin Response to Hypotension\(^a\)

<table>
<thead>
<tr>
<th>Perfusion Period(^b)</th>
<th>Perfusion Pressure (cm H(_2)O)</th>
<th>Renin Activity (ng Al/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>19.2 ± 1.35(^c)</td>
<td>1.61 ± 0.36(^d)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>Second</td>
<td>14.2 ± 1.20(^c)</td>
<td>3.35 ± 0.48(^d)</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

\(^a\) All values are means ± S.E.M. (N)

\(^b\) Initial refers to the first 90 minutes of perfusion and second refers to the second 90 minutes of perfusion

\(^c\) \(P<0.01\)

\(^d\) \(P<0.05\)
plasma renin activity and indeed there was found an inverse correlation between plasma levels of propranolol and plasma renin activity (Forman and Mulrow, 1974). Stress-induced renin release may also be inhibited by propranolol (Leenen and Shapiro, 1974). These studies were concerned primarily with the effects of the sympathetic system on renin release and generally do not involve the baroreceptor response. In a more recent study carried out by Osborn et al. (1977), it was in the denervated non-filtering kidney model, beta blockage could blunt furosemide induced renin release although the response could not be entirely abolished. Therefore, in mammalian species it would appear that the baroreceptor response may in part be mediated via the sympathetic nervous system, specifically the beta receptors.

This does not appear to be the case in trout for as previously mentioned beta receptor blockage had no major effect on the baroreceptor response. It is possible that the alpha and beta receptors may have a secondary effect, possibly as a result of an action on vascular tone. This would explain the considerable disparity in the levels of renin activity between the two experiments, control 0.02 ng Al/ml/hr for alpha and 1.61 ng Al/ml/hr for beta at similar perfusion pressures while following a decline in perfusion pressures the values were 0.1 ng Al/ml/hr for alpha and 3.35 ng Al/ml/hr for beta, again at similar perfusion pressures. If vascular tone does exist in fish kidney, then while the drugs would not have any direct effect on the JG cells, there may be an effect as a result of the loss of vascular tone due to the blocking agents. Accordingly the next experiment was to block both receptors and observe the effect on renin release.

Figure 3.8 demonstrates the pattern of renin activity, and consequently renin release, over the total perfusion period when both alpha and beta re-
Figure 3.8. The effect of combined blocking agents on renin release caused by changing renal perfusion pressure in the isolated perfused trout kidney. The y-axis represents renin release as measured by perfusate renin activity and is expressed in ng AI/ml/hr. The x-axis represents perfusion period and is measured in minutes. The break in the line between 90 minutes and 100 minutes is the point at which the perfusion pressure head was lowered. Each point on the line represents the mean ± S.E.M. of 9 determinations. In this case the dosage of the alpha blocking agent phenoxybenzamine was $2 \times 10^{-5}$ gm/ml perfusate and the beta blocking agent propranolol was $10^{-6}$ gm/ml perfusate.
TABLE 3.4

Effect of Combined Alpha and Beta Receptor Blockage on Baroreceptor Induced Renin Secretion

<table>
<thead>
<tr>
<th>Perfusion Period</th>
<th>Perfusion Pressure (cm H$_2$O)</th>
<th>Renin Activity (ng AI/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>$22.2 \pm 0.87^c$</td>
<td>$0.191 \pm 0.034^d$</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>Second</td>
<td>$14.2 \pm 1.27^c$</td>
<td>$0.52 \pm 0.06^d$</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

a - All values are means ± S.E.M. (N)
b - Initial refers to the first 90 minutes of perfusion while second refers to the second 90 minutes of perfusion
c - P<0.01
d - P<0.05
ceptors were blocked. Up to 60 minutes of the initial (high pressure) perfusion period renin release rose slightly, this being followed by a small decrease. When the perfusion pressure declined from an average of 22.2 cm H₂O to 14.2 cm H₂O (36%), renin activity increased from 0.191 ng AI/ml/hr to 0.52 ng AI/ml/hr, a nearly 3 fold increase. The levels of renin activity obtained in this study appear to be approximately midway between those obtained when only one blocking agent is used. This experiment serves to confirm the hypothesis that the sympathetic receptors do not play a significant role in the response to a decline in renal perfusion pressure because blocking both receptor types had no discernible effect on the hypotension induced renin release. This was to be expected as blocking either receptor type singly had no effect. But the levels of renin activity obtained in this experiment were similar to those found in the initial experiments in this series (see Figures 3.3 and 3.4 and Table 3.1). Thus there may exist some sort of vascular tone in the renal circulation which is modified by action on either the alpha or beta receptors. But this is somewhat speculative at this time as there is little information available on the nature of the vascular bed in the fish kidney. It is equally a possibility that these variations in overall levels may simply reflect a seasonal variation or changes in some other uncontrolled variable, as these experiments were carried out over a 6-month period.

Since these receptors do not play any significant role in the release of renin, caused by hypotension, the decline in perfusion pressure must affect the J.G cells in some direct fashion. As the J.G. cells are part of the arteriole it is probable that when renal perfusion pressure decreases the transmural pressure seen by these cells decreases. Thus the degree of stretch sensed by these cells changes. This in turn results in a deformation of
these cells followed by the release or active secretion of renin from the cell. The secretion of renin following the membrane deformation may be an active process which is partially calcium dependent (Baumbach and Leyssac, 1977). But extracellular calcium does not play an essential role in the mechanics of renin secretion although it may be necessary for synthesis and/or storage (Lester and Rubin, 1977). More recently, Park and Malvin (1978) found that renin secretion is mediated in part by intracellular changes in calcium concentration. While these experiments were done on mammalian subjects it does not appear unlikely that a similar mechanism could be found in fishes.

3.5.4 Inhibition of Renin Secretion.

Once renin secretion has been initiated, what is the signal which causes an inhibition of renin secretion? There are two possibilities, one being a rise in perfusion pressure and the other being a hormonal negative feedback system. The former would be a result of the action of renin in causing a vasoconstriction and a rise in perfusion pressure. The latter involves again the formation of angiotensin II but this time the generated hormone feeds back to inhibit further renin release from the J.G. cells. Angiotensin I could be the compound which inhibits further release but it is more logical to assume that it is the end-product which is the inhibitor, presupposing the existence of a negative feedback system of this type.

Figure 3.5 and Table 3.1 illustrate the effect of increasing perfusion pressure on renin and as previously noted, the increased perfusion pressure had no significant effect on renin activity and consequently renin release. However as may be seen in Figure 3.9, incubation of kidney slices with angiotensin II results in a steady dose-dependent decline in renin secretion from these kidney slices. Therefore, since this is an **in vitro** situation and
Figure 3.9. The \textit{in vitro} effect of angiotensin II on renin release. The y-axis represents the renin release as measured by renin activity and is expressed in ng AI/ml/hr. The x-axis represents the dose of angiotensin II added to the incubation medium and is in ng. For the line equation $y = -0.005x + 1.62$ the correlation coefficient $r = -.88$ is significant at the 1% level.
there is no longer an intact renal vascular bed, the decreased renin activity is a result of a direct effect on the JG cells by the angiotensin II. Thus renin secretion is inhibited in a short-loop negative feedback system involving angiotensin II.

This system is not unique to fishes and has been found in various mammalian species. Vander and Geelhoed (1965) found that renin secretion in dogs could be inhibited by low doses of angiotensin II. This phenomenon may also be observed in sheep (Blair-West et al., 1971), humans (DeChamplain et al., 1966) and rats (Vandogen et al., 1974). In addition, blocking angiotensin II by use of specific angiotensin antagonists such as 1-Sarcosine, 8-Alanine, angiotensin II and converting enzyme inhibitors results in an increase in plasma renin activity in all species examined. These data are consistent with the hypothesis that renin secretion is inhibited by a short-loop negative feedback type mechanism (Davis and Freeman, 1976).

3.5.5 Model Mechanism for Renin Secretion.

Based on the experimental evidence previously discussed the following model for renin secretion in teleosts is proposed. A decline in renal perfusion pressure results in a decrease in the transmural pressure in the afferent arterioles. This causes a deformation of the J.G. cells which are located in the walls of the afferent arterioles and there is a consequent secretion of renin. The circulating renin then acts on the renin substrate in the blood plasma to form angiotensin I which is converted into angiotensin II. This angiotensin II thus formed exerts two effects. One is on the arterioles causing vasoconstriction which probably has little or no effect on the release of renin. The other is a direct effect on the J.G. cells to inhibit further renin release in a short-loop negative feedback type mechanism. The evidence which supports this hypothesis that renin release is a
result of a direct effect on the JG cells is the lack of effect of either alpha or beta receptor blockage. The *in vitro* inhibition of renin secretion by the addition of angiotensin II to the incubation medium supports the negative feedback hypothesis as does the lack of effect of increasing renal perfusion pressure.

3.5.6 Summary.

In summary, renin is secreted in response to a decline in renal perfusion pressure. This is not mediated by the sympathetic nervous system. Renin secretion is inhibited in a short-loop negative feedback type mechanism.
Section IV Introduction.

4.0 SODIUM AND RENIN RELEASE.

4.1 Macula Densa Hypothesis.

4.1.1 Relationship of Macula Densa and JG Cells.

The macula densa is an area of specialized cells in the distal convoluted tubule of mammalian nephrons (Ham, 1965), as is diagrammatically illustrated in Figure 4.1. This area appears to play a role in renin release but the nature of that role has to this time been somewhat controversial. The cells of the macula densa are highly specialized cells in close contact with the granular juxtaglomerular (JG) cells (Hartroft and Hartroft, 1961). In fact, these cells are only separated from the JG cells by an incomplete basement membrane with cytoplasmic extensions (Barajas and Latta, 1967). In addition, there is a positive correlation between glucose-6-phosphate dehydrogenase activity in the macula densa and renal renin or JG cell granulation in several experimental conditions in rats such as sodium-deficient diet and adrenalectomy (Capelli et al., 1968).

Barajas (1971) has found that the macula densa is more closely associated with the afferent arteriole and mesangial cells than with the JG cells in the rat. This author hypothesized that the degree of contact between the macula densa and the JG cells is a function of tubular volume and sodium load and that as sodium and tubular volume decrease the distance between the macula densa and the JG cells increases. This then results in an increase in renin secretion.

Further evidence which relates the macula densa to the JG cells is provided by the work of Vander and Miller (1964) who found that renin secre-
Figure 4.1. Diagrammatic representation of the juxtaglomerular apparatus of mammals. AA - afferent arteriole, EA - efferent arteriole, G - glomerulus, RG - renin granules, MD - macula densa, DCT - distal convoluted tubule, ECM - extraglomerular mesangium.
tion after aortic constriction could be blocked by simultaneous administration of diuretics. Also renin secretion can be inhibited by hypernatremia and hyperkalemia in the normal intact filtering kidney but not in the non-filtering kidney model. This leads to the conclusion that the macula densa is related to the J.G. cells in some fashion as the macula densa has been rendered non-functional in the non-filtering kidney and renin secretion is not inhibited by either hypernatremia or hyperkalemia (Shade et al., 1972).

Retrograde microperfusion studies have provided other evidence of the relationship between the macula densa and the J.G. cells. Retrograde perfusion of the macula densa with solutions of sodium chloride has provided two pieces of evidence; one being that renin activity in a single J.G. apparatus was increased and the other being that single-nephron glomerular filtration rate decreased which could be attributed to afferent arteriolar constriction due to increased local formation of angiotensin II (Thurau, 1971; Thurau et al., 1972).

4.1.2 Role of the Macula Densa in Renin Release.

Having established that there is a relationship between the macula densa and the J.G. cells, the next problem to confront is what is the role of the macula densa in renin release, i.e. to what does the macula densa respond? Administration of diuretics blocks the renin response to aortic constriction and it was suggested that the blockage was due to increased sodium load at the macula densa caused by the diuretics (Vander and Miller, 1964). They then suggested that a decrease in macula densa sodium leads to an increase in renin secretion. A low sodium intake results in sodium and volume depletion, decreased glomerular filtration rate, and, in severe cases, hyponatremia so that the filtered load of sodium is decreased with a concomitant increase in plasma renin activity (PRA) in man (Brown et al., 1963; 1964).
Similar effects have been shown in other mammalian species, notably the rat and the dog (Vander and Luciano, 1967; Pickens and Enoch, 1968; Vander and Carlson, 1969).

While it would appear that a low sodium load at the macula densa causes renin secretion as shown by the previous studies, Davis and Freeman (1976) in their recent review have suggested that these studies may not be as definitive as once thought. They point out that to extrapolate the value for filtered sodium to the concentration of sodium at the macula densa, i.e., macula densa sodium load, may not be valid as there is the additional influence of the loop of Henle on tubular sodium and chloride before it reaches the macula densa. This area is rather inaccessible to micropuncture and so little is known about the changes that occur in this area. Also, these authors point out that there is evidence for a chloride pump rather than a sodium pump in the ascending limb of the loop of Henle.

Another group of workers have held to the hypothesis that it is an increased sodium load which the macula densa senses (Thurau et al., 1967; Thurau et al., 1972). This group used retrograde perfusion of the nephron and observed that isotonic or hypertonic sodium solutions injected into the distal tubule resulted in either a decrease in proximal tubule diameter or proximal tubule collapse. These observations were interpreted as a result of reduced glomerular filtration rate. Control experiments conducted with hypotonic sodium chloride or with hypertonic and/or isotonic choline chloride and mannitol solutions did not show any changes in proximal tubule diameter. They then proposed the following mechanism: an increased release of renin in response to increased sodium concentration at the macula densa, local angiotensin II formation in the afferent arteriole, afferent arteriolar constriction, decreased glomerular filtration rate and thus proximal tubule.
collapse. Thurau (1971) originally proposed that the increased sodium load caused or resulted in an increase in renin release. However, in a later paper (Thurau et al., 1972) it was maintained that only increased renin activity in the J.G cells occurred and this reflected increased renin activation from a preformed molecule. This was due to the fact that the time course of the response to the retrograde perfusion was too rapid to allow for renin synthesis.

This hypothesis has been supported by a number of other groups. Rabbits given furosemide showed an increase in PRA despite the fact that sodium and volume depletion were prevented by reinfusing ureteral urine into the femoral veins (Meyer et al., 1968). As the ascending limb of the loop of Henle is the major site of action of furosemide (Clapp and Robinson, 1968) and the concentration of sodium in the early part of the distal tubule is increased, Meyer et al. (1968) suggested that the increased sodium concentration at the macula densa was causing an increase in renin secretion and consequently the increased PRA. Cooke et al. (1970) found that intravenous infusion of ethacrynic acid during reinfusion of ureteral urine into the femoral veins caused an increase in renal vein renin activity while chlorothiazide did not. Since chlorothiazide acts on the distal convoluted tubule while ethacrynic acid acts on the ascending limb of the loop of Henle, they suggested that a sodium rich tubular fluid was reaching the macula densa in the first case but not in the second. It was then proposed that the increased sodium load was causing the increase in renal vein renin activity. This response has also been observed in another study (Birbari, 1972). In vitro studies have also supported the Thurau hypothesis in that increasing sodium concentrations result in increased renin secretion while the addition of oubain, a sodium-potassium ATPase inhibitor, causes a decrease in renin secretion under these
conditions (Lyons and Churchill, 1974). Another group, Blair-West et al. (1977), observed that dehydrated sheep had plasma renin contents two to three fold greater than normal levels. They found that changes in plasma renin content were related to sodium consumption, excretion and plasma content but not to plasma volume insofar as plasma volume is reflected by alterations in plasma proteins. They concluded that the changes in renin release were mediated by the increased sodium levels and not by alterations in body fluid volume and were under the influence of the macula densa mechanism.

This hypothesis has been attacked by a number of other workers in the field. Gottschalk and Leyssac (1968) pointed out that the proximal tubular collapse could be due to fluid leaking from sites where the proximal tubule was punctured for injection of the lissamine green dye in the Thurau preparation. Also the proportion of angiotensin I to angiotensin II conversion in the kidney appears to be low and this is further evidence against the Thurau hypothesis (Vane, 1974). In addition Davis and Freeman (1976) noted that the observation that choline chloride failed to influence the mechanism proposed by Thurau must be reconciled with the evidence for an active chloride pump in the ascending limb of the loop of Henle. This latter criticism may not be valid as Stephens et al. (1978) have found that sodium or potassium lactate inhibited renin secretion in the dog. This evidence supports the theory that it is sodium levels at the macula densa which affect renin and not chloride levels. Corsini et al. (1974) have noted that in vitro studies may not necessarily be giving useful information as renin secretion by the kidney slices in vitro may be proceeding at a maximal or near-maximal rate and thus respond only weakly at best to stimuli which would usually elicit renin release in vivo.
The original hypothesis of Vander and Miller (1964) was that increased sodium load at the macula densa leads to a decrease in renin secretion. This hypothesis also has been supported by a number of other groups. Freeman et al. (1974) found that when renin secretion and renal vein renin activity were compared during infusion of ethacrynic acid there was a marked decline in renin secretion but not in renal vein renin activity. This decline in renin secretion was associated with a marked increase in urinary sodium concentration and rate of secretion. Thus a high tubular sodium level inhibited renin secretion but not the activity of prereleased renin. DiBona (1971) found that renin secretion increased in association with a decreased distal tubular sodium delivery and concentration which supports the original Vander and Miller hypothesis. Intravenous infusion of sodium chloride or sodium sulfate revealed that sodium sulfate inhibited renin secretion to a greater extent than sodium chloride which suggested that it was sodium levels rather than sodium and chloride which inhibited renin release (Churchill et al., 1975). This was confirmed by the work of Stephens et al. (1978) who used sodium lactate to inhibit renin release. However, Kotchen et al. (1978) found that choline chloride inhibited renin to a greater extent than sodium chloride while choline bicarbonate was totally without effect. These results suggested that chloride may have a very important role to play in regulating responses to sodium loading in the rat. Nash et al. (1968) presented evidence for a sodium-sensitive mechanism located in the kidney and suggested that the signal which mediated renin release was a result of decreased sodium transport by the macula densa cells. Vander and Carlson (1969) explained that the effect of furosemide on renin release is probably due to a direct action of the drug on the cells of the macula densa; this action being to decrease sodium transport by the macula densa.
4.1.3 Summary.

In summary, there is an area of specialized cells in the distal convoluted tubule of the nephron of mammals which is called the macula densa. This area is anatomically and functionally related to the JG cells and plays a role in renin release from the JG cells. The nature of the signal to which the macula densa responds is somewhat controversial. There are two main hypotheses, the Thurau hypothesis which states that an increased tubular sodium load at the macula densa causes an increase in renin release or renin activation and the Vander hypothesis which claims that the macula densa stimulates renin release only when faced by a decrease in tubular sodium concentration. At this time it is not possible to reconcile these two hypotheses as both are backed by good experimental evidence and each may explain away the effects of the other.

4.1.4 Macula Densa in Non-Mammalian Vertebrates.

As was shown previously, the macula densa is an important part of the juxtaglomerular apparatus in mammals and this leads to the question is such a structure present in non-mammalian vertebrates? Sokabe and Ogawa (1974) have reviewed the evolution of the juxtaglomerular apparatus in vertebrates. It appears that a structure homologous or analogous to the macula densa is not found in fishes, amphibians or reptiles. However, there is a structure in the distal convoluted tubule of birds which resembles the macula densa of mammalian species. Examination by electron microscopy reveals that these cells do not possess all of the characteristics of the mammalian type and therefore are probably an intermediate of some sort as the bird kidney is generally considered to be intermediate between that of the reptiles and of mammals.
Since a macula densa is lacking in the lower vertebrates, is the renin response to changing sodium loads equally lacking? Sokabe et al. (1972) have examined PRA in bullfrogs which were in various dehydrated states and found that dehydration decreased PRA while intravenous infusion of isotonic saline or 2% glucose resulted in an increase in PRA. This response then appears to be a response to changing plasma osmolarity rather than a sodium response. However, where marine teleosts were adapted to a dilute medium, PRA declined in American eels but not in toadfish (Nishimura et al., 1976). Sokabe et al. (1973) found that PRA showed a transient increase when Japanese eels were transferred from freshwater to seawater. When *Tilapia mossambica* were adapted to seawater, it was observed that the number of JG cells in the kidney increased over those in freshwater adapted fishes and appeared to be larger in size (Krishnamurthy and Bern, 1973). On the other hand, Malvin and Vander (1967) did not observe any changes in plasma renin in two species of tuna and *Tilapia mossambica* when these fish were adapted to a dilute medium despite changes in plasma sodium. Therefore, the evidence for a renin response to changing sodium levels, of various fish species, is at best controversial.

4.1.5 Sodium as a Humoral Agent.

Sodium may act as a humoral agent in various mammalian species. Hartroft and Hartroft (1961) were the first to suggest that plasma sodium levels influence renin secretion from the JG cells. The most convincing evidence for sodium acting as a humoral agent comes from a series of experiments which involved altering sodium concentrations in the plasma perfusing the isolated dog kidney (Yamamoto et al., 1968). Another group found that intrarenal infusions of hypertonic saline to dogs with a non-filtering kidney had no effect on renin secretion for the first 45 minutes of infusion but then a
significant decrease in renin secretion occurred after 60 minutes of infusion which most probably reflects a secondary mechanism (Shade et al., 1972). Saline loading of dogs decreases renin activity (Churchill et al., 1974), while dehydrated sheep show an increase in PRA about 2-3 times greater than control levels (Blair-West et al., 1977). In both cases the authors concluded that this was due to a macula densa mechanism. Therefore the evidence for sodium acting as a humoral agent is scanty and it is quite probable that such an effect would not function in the normal physiology of mammals as the macula densa mechanism is more than adequate.
Section IV Materials and Methods.

4.2 Sodium Perfusion of Kidney.

4.2.1 Non-Filtering Kidney.

Adult trout were prepared for kidney perfusion as was previously described. The perfusion apparatus was modified by setting up two perfusion bottles and reservoirs connected by a 3-way polyethylene stopcock so that perfusion fluids could be changed without altering perfusion pressure. The kidney was perfused with Cortland saline, sodium concentration 138 meq/l, for 90 minutes post-clearance time and samples collected as described in the preceding chapter. The stopcock was then switched so that the kidney was being perfused with a hypertonic sodium Cortland saline (198 meq Na⁺/l) for a further 90 minutes, samples being taken for every 30 minutes.

These perfusate samples were then analyzed for renin activity by adding 0.5 ml sample to 0.5 ml homologous plasma, vortex mixing and then using the radioimmunoassay procedure.

4.2.2 Filtering Kidney Preparation.

A kidney perfusion preparation was modified in two respects to create a filtering kidney preparation. These were as follows: first the caudal vein as well as the caudal artery was cannulated with Intramedic PE 60 so as to perfuse a portion of the renal portal circulation of the kidney and second the ureters were not tied off but rather cannulated with a length of Intramedic PE 90 to allow urine collection.

The preparation was then perfused for 90 minutes (post-clearance time) with Cortland saline and perfusate samples collected. In addition urine samples were collected in a disposable micro-pipet for the first 30 minutes
of perfusion and the last 30 minutes of perfusion. Hypertonic sodium Cortland saline was then used to perfuse the kidney for a further 90 minutes, perfusate samples and urine samples were collected for the first 30 minutes and the last 30 minutes in disposable micro-pipets. The pipets were then sealed with clay and stored frozen for later analysis.

Perfusate samples were then assayed for renin activity and urine flow rates calculated from the urine volumes collected.

In addition the kidney was examined to determine if the perfusate was actually passing through the arterial and venous circulation. This was carried out by means of the Batson's corrosion casting technique as described by Murakami (1971) and Nowell et al. (1972) and Gannon (1974). Separate casts were done to determine the extent of the arterial and venous circulation but in each case the compound was injected through the respective cannula until it was observed to appear in the heart cannula.

### 4.2.3 Perfusion Solutions

Two types of perfusion solutions were utilized during this study. The first was Cortland saline with 2% PVP added and secondly Cortland saline with additional sodium in the form of sodium chloride and 2% PVP. This latter solution is referred to as hypertonic sodium Cortland saline. In both cases chlorophenol red (B.D.M. Canada) was added to the perfusate in the concentration of 250 mg/l. The presence of chlorophenol red in the urine was taken as evidence that the tubular cells of the kidney were viable and capable of active transport as this dye is known to be actively transported by teleost nephrons (Hickman and Trump, 1969).
4.2.4 Statistical Methods.

The Student's t test was used throughout this series of experiments to test the significance of a difference between two means. A P value of less than 0.05 was considered significant and a P value of less than 0.01 was considered highly significant.
Section IV Results and Discussion.

4.3 Renin Release Following Sodium Perfusion in Trout Kidneys.

4.3.1 Non-Filtering Kidney.

Hypertonic saline perfusion of the isolated non-filtering kidney has little or no effect on renin release as may be seen in Figure 4.2 and Table 4.1. The renin activity oscillates up and down over the entire perfusion period, both during isotonic and hypertonic saline perfusion. At the end of the total perfusion period renin activity has shown a slight increase above the beginning level. Table 4.1 shows that the average renin activity of 0.07 ng AI/ml/hr obtained during the isotonic saline perfusion has increased slightly to 0.074 ng AI/ml/hr during the hypertonic saline perfusion.

The purpose of these experiments was to determine if plasma sodium exerted a direct humoral effect on renin release in the trout. The data indicate that there is no such effect since the renin activity obtained in these experiments did not show any significant trend following hypertonic saline perfusion with constant perfusion pressure.

But dehydration of fish causes a significant increase in plasma renin activity (Sokabe et al., 1966; Sokabe et al., 1968) and this increase may be mediated by changes in sodium concentration. Although plasma sodium has no effect on renin secretion there still exists the possibility that tubular sodium loads may affect the rate of renin release from the J.G. cells.

4.3.2 Filtering Kidneys.

In this preparation the kidney is intact and capable of both passive filtration and active transport as is evidenced by the fact that chlorophenol red from the perfusate appears in the urine. Figure 4.3 shows the pattern of
Figure 4.2. The effect of hypertonic saline perfusion on renin release from the isolated perfused non-filtering trout kidney. The y-axis represents renin release as measured in terms of perfusate renin activity, and is in ng AI/ml/hr. The x-axis is time of perfusion and is in minutes. The first 90 minutes of perfusion represent the period of isotonic saline perfusion. The break in the line between 90 and 100 minutes represents the time at which the perfusion solutions were changed and the last 90 minutes of perfusion, time 100 to time 190, represent the period of hypertonic saline perfusion. Each point is the mean ± S.E.M. of 10 determinations.
### TABLE 4.1

**Effect of Sodium Perfusion on Renin Release in Non-Filtering and Filtering Kidneys**

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>Non-Filtering</th>
<th>Filtering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic Saline</td>
<td>0.07 ± 0.02</td>
<td>1.4 ± 0.113</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(7)</td>
</tr>
<tr>
<td>Hypertonic Saline</td>
<td>0.074 ± 0.014</td>
<td>1.22 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

*a - All values are means ± S.E.M. (N) of renin activity in ng AI/ml/hr*
Figure 4.3. The effect of hypertonic saline perfusion on renin release in isolated perfused filtering trout kidney. The y-axis represents renin release as measured in terms of perfusate renin activity in ng Al/ml/hr. The x-axis is time of perfusion and is in minutes. The first 90 minutes of perfusion represent the isotonic saline perfusion, and the break in the line between 90 and 100 minutes is the time at which the perfusion solutions were changed. The remaining 90 minutes represent the period of hypertonic saline perfusion. Each point is the mean ± S.E.M. of 7 determinations.
renin secretion during isotonic saline perfusion and hypertonic saline perfusion. Renin activity shows an approximately 10% decrease during the hypertonic saline perfusion at one point but following this appears to be increasing back to the control level. This decrease is not significant. Table 4.1 shows the average effect of both perfusions on renin secretion and as may be seen renin activity shows a slight non-significant decrease from the control level.

The macula densa is the tubular receptor for sodium loads in the mammalian kidney (Davis and Freeman, 1976) but this structure may be peculiar to mammals as it is not found in fish, amphibians, or reptiles but may exist in a rudimentary form in birds (Sokabe and Ogawa, 1974). Therefore one may expect that sodium would not exert any effect on renin secretion in non-homeothermic vertebrates and this appears to be so in fishes as there was no significant change in renin secretion following hypertonic saline perfusion of the filtering kidney preparation.

The decline in renin activity observed during the hypertonic saline perfusion appears to be a continuation of the decline observed during the isotonic saline perfusion period. Since surgical trauma and/or stress stimulate renin release in mammalian species (Reid et al., 1978), it is possible that a similar situation exists in fish and that the observed decline is a result of abnormally high trauma-induced renin release. While it is possible that such an effect, if present, could mask a renin response to the high tubular sodium levels obtained during hypertonic saline perfusion (see Appendix 1), this is unlikely. Other authors have found that inhibition of renin release by one set of circumstances may be overridden by a stimulus which causes renin release (Churchill et al., 1974). The fact that the observed decline in renin secretion is only 10%, that it appears to be part
of an overall trend and as well renin activity seems to be on the rise at the end of the hypertonic sodium perfusion period argues against masking of a sodium effect. Therefore the conclusion which may be drawn from these experiments is that sodium has no effect on renin release in the trout.

4.3.3 Summary.

In summary, sodium perfusion of the non-filtering trout kidney has no effect on renin secretion. Hypertonic saline perfusion of filtering trout kidneys is equally without effect. Therefore the conclusion drawn is that renin secretion in the rainbow trout is probably not directly affected by either plasma or tubular sodium levels.

4.4 Sodium Perfusion and Urine Flow Rates.

One of the initial responses to a transition from freshwater to seawater in euryhaline species is a decline in the GFR and urine flow rates (Oide and Utida, 1967). Various groups have attempted to explain this immediate response of the kidneys as being mediated by various humoral agents or by some form of neural control. Table 4.2 shows the effect on urine flow rates when perfusing the filtering kidney preparation. Urine flow undergoes a significant decrease, from 0.163 ml/kg/hr to 0.097 ml/kg/hr. During each perfusion the urine flow decreases slightly, i.e., during isotonic saline, 0.163 to 0.157 ml/kg/hr, and hypertonic saline, 0.097 to 0.07 ml/kg/hr, and this is not a significant decrease. The overall decrease in flow is significant indicating that the increased sodium or more probably osmotic pressure causes a decrease in flow rates. Since the bladder has been removed from consideration, as the ureters are cannulated, the urine flow rate reflects the glomerular filtration rate. These data indicate that an increased plasma sodium load will cause a decrease in the glomerular filtration rate. How-
TABLE 4.2

Effect of Hypertonic Saline Perfusion on Urine Flow Rates in the Isolated Perfused Kidney$^a$

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>Begin Perfusion</th>
<th>End Perfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic Saline</td>
<td>0.163 ± 0.01$^b$</td>
<td>0.157 ± 0.011$^c$</td>
</tr>
<tr>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Hypertonic Saline</td>
<td>0.097 ± 0.019$^b$</td>
<td>0.07 ± 0.02$^c$</td>
</tr>
<tr>
<td>(7)</td>
<td>(7)</td>
<td></td>
</tr>
</tbody>
</table>

*a - All values are means ± S.E.M. (N) and units are ml/kg/hr

b - P<0.01

c - P<0.01
ever, further work is required to determine if this is a direct effect of the sodium ion or an effect due to increased osmotic pressure of the perfusate which would lead to an increased fluid (water) reabsorption from the nephron.
Section V Introduction.

5.0 ANGIOTENSIN AND THE KIDNEY.

5.1 Mammals.

The renal effects of the renin-angiotensin system may be divided into direct effects and indirect effects. That is, the effect of angiotensin on the renal vasculature and tubule and effects mediated by another hormone which is liberated from another gland by the action of angiotensin.

5.1.1 Direct Effects of Angiotensins.

Angiotensin I (AI) has long been regarded as the inactive precursor of angiotensin II (AII) although in isolated myocardial preparations it displayed approximately 50% of the activity of AII. However the activity could be abolished by pretreatment of the preparation with converting enzyme inhibitors obtained from Bothrops jararaca venom which indicates that its activity on myocardial preparations is due to conversion to AII (Peach, 1977). Infusions of AI into the perfused kidney in the presence or absence of a converting enzyme inhibitor induce a selective decrease of the inner cortical and medullary blood flow without apparently affecting the outer cortical blood flow. AII consistently decreased the outer cortical blood flow but had variable effects on the inner cortical flow (Itskovitz and McGriff, 1974; Vane and McGriff, 1975). These authors have proposed that AI may be the major determinant of the partitioning of the intrarenal blood flow. Osborn et al. (1974) found that AI could alter renal blood flow in sheep but that AII had a much greater effect. In this study, it was not determined if AI had a direct effect or one mediated by conversion of AI to AII.

AII on the other hand is known to have both diuretic and antidiuretic
actions as well as natriuretic and antinatriuretic actions on the mammalian kidney. It is known that AII is a vasoconstrictor (Sokabe, 1974) and it is this action which has been used to explain the often conflicting effects of AII on the kidney. Small doses cause both diuresis and natriuresis in rat, rabbit, and dog (Healy et al., 1965; Langford and Pickering, 1965; Cannon et al., 1966; Barraclough et al., 1967; Malvin and Vander, 1967), while Davis et al. (1974) consider that AII acts directly on the afferent arterioles to reduce renal blood flow to aid in maintenance of systemic blood pressure.

Since renin is found in the kidney and mammalian kidneys are known to autoregulate blood flow, i.e., keep renal blood flow constant over a wide range of pressures, there is a possibility that angiotensin may be involved in this autoregulatory process (Fojas and Schmid, 1970; Gagnon et al., 1970). More recent work has resulted in a certain amount of controversy. Potkay and Gilmore (1973) found that renal autoregulation was unaffected by renin depletion in dogs. Also, renal autoregulation was maintained even when AII was not present (Gagnon et al., 1974). Sokabe (1974) does not believe that the renin-angiotensin system is involved with renal autoregulation as renin is released in situations which would call for a vasodilation if autoregulation is to be maintained. However, when dogs are maintained on desoxycorticosterone (DOC) and a high sodium diet to deplete the kidneys of renin, renal autoregulation is impaired across a large range of either high or low arterial pressures (Kaloyanides et al., 1974). This supports a similar study by Brech et al. (1973) who found that renal autoregulation and distribution of renal blood flow were severely affected in dogs which were kept on a high sodium diet and DOC which causes renin depletion. Thus there are arguments both for and against a role of the renin-angiotensin system in renal autoregulation and further work is required to settle this controversy.
Recent work has shown that AII may have a direct effect on the renal tubules. Melton and Frazier (1976) observed that angiotensin infused into the left kidney of dogs produced consistent reductions in the excretion of sodium, potassium and chloride. These changes could not be attributed to alterations in glomerular filtration rate or renal plasma flow. Electrolyte excretion by the right uninfused kidney was constant. These data are consistent with the hypothesis that AII may function as an intrarenal antinatriuretic hormone. This hypothesis is supported by the work of Johnson and Malvin (1977) who observed that AII exerts a direct stimulatory effect on renal tubular sodium reabsorption independent of changes in glomerular filtration rate, renal plasma flow, filtration fraction, or intracortical distribution of blood flow.

5.1.2 Indirect Effects of Angiotensins.

The indirect effect of AII on the kidney involves sodium retention and is mediated by aldosterone. AII stimulates the first step in the biosynthesis of aldosterone, the conversion of cholesterol to pregnenolone (Aguilera and Marusic, 1971). It would appear that it has no effect on the conversion of corticosterone to aldosterone (Haning et al., 1971) and the stimulatory effect of AII on the zona glomerulosa of the adrenal cortex may be inhibited by either high sodium or low potassium concentrations (Dufau et al., 1969; Boyd et al., 1973). Further work has shown that the stimulus for aldosterone biosynthesis may not be AII but rather a naturally occurring metabolite, des-aspartyl-AII, which has been tentatively called angiotensin III (Campbell et al., 1974). Williams et al. (1974) have observed that AII is less active than angiotensin III (AIII) in the adrenal cortex. These authors have suggested that AII binds to the zona glomerulosa receptor where it is converted to the heptapeptide which then acts on the cells to stimulate aldosterone
biosynthesis. On the other hand, more recent work has tended to disagree with this hypothesis and has found that in the dog, AII was not converted to AIII in isolated zona glomerulosa cells and that aldosterone was still produced (Douglas et al., 1978).

AII has been implicated in the release of ADH in rats (Claybaugh and Share, 1972). This work has also been duplicated by other groups (Keil et al., 1975; Gregg and Malvin, 1978).

5.1.3 Summary.

In summary, AII has both direct and indirect effects on the kidney. The direct effects include an alteration in renal blood flow, an antidiuretic effect and a diuretic effect as a result of the vasopressor function. Other direct effects are natriuretic or antinatriuretic functions which may be a result of an effect on the renal tubules. In addition, AII has indirect effects on the kidney which are mediated by some other hormone(s) released by the action of AII at their sites of origin.
5.2 Renal Effects of Angiotensins.

5.2.1 Perfusion Solutions.

Perfusion solutions consisted of Cortland saline with 2% PVP. The solution used to perfuse the venous circulation of the kidney also contained chlorophenol red (B.D.H. Canada Ltd.) at a concentration of 250 mg/l. A series of angiotensin II (Hypertensin - CIBA) solutions were made, each containing either 50, 100, 150, 200, or 250 ng angiotensin II/ml and these were used for injection purposes. A similar series of angiotensin I (Calbiochem) solutions was used for injection purposes; the concentrations were identical with the angiotensin II solutions. The angiotensins, both I and II, were dissolved in Cortland saline to avoid any possible osmotic effects.

5.2.2 Angiotensin Injections.

The kidney of adult trout of either sex was prepared for perfusion as previously described. This preparation was of the filtering kidney type thus allowing the effects of either angiotensin I or II on urine flow rate to be determined.

To determine the effects of angiotensin I on urine flow rates, two series of experiments were carried out. The protocol was as follows. Urine flow rate was measured for 30 minutes (post-clearance time) with constant perfusion pressure. A 1 ml dose of an angiotensin I solution was injected via the perfusion line and urine flow rate measured for another 30 minutes. A second angiotensin I dose was injected, urine flow measured, and the procedure repeated until one dose of each of the standards had been injected. In the first series of experiments the angiotensin was injected into the arterial perfusion line and in the second series it was injected into the
venous perfusion line.

The effects of angiotensin II on urine flow rates were determined by using the same protocol as described above except that angiotensin II was injected.

In all experiments urine was collected in glass disposable micro-pipets (Becton, Dickinson and Company), the ends sealed with plastic clay (Clay-Adams, Inc.) and stored frozen until later analysis. The urine was protected from contamination by the clay by allowing a small air bubble to remain at each end of the pipet between the surface of the fluid and the clay. The presence of chlorophenol red in the urine indicated that the kidney preparation was viable. If chlorophenol red did not appear the preparation was discarded.

5.2.3 Ion Analysis.

The frozen urine samples were thawed, the ends of the micro-pipets cut off and the sample blown gently onto a piece of Parafilm (American Can Co.) where the sample formed a small bubble. Aliquots of each sample were quickly taken and diluted 1:1,000 with distilled water and analyzed for sodium concentration by flame emission spectrophotometry on a flame photometer.

For urinary potassium analysis, aliquots of the urine were diluted 1:1,000 by a 500 meq/l Na+ solution. This "sodium-swamping" technique allows any interference by sodium to be cancelled out when calibrating the machine. Urinary potassium levels were then measured by flame emission spectrophotometry using a flame photometer.

5.2.4 Statistical Methods.

Linear regression analysis was used to determine the correlation coefficient (r) while the Student's t test was used to compare means. In each
case a P value of less than 0.05 was considered significant and a P value of less than 0.01 was considered highly significant.
Section V Results and Discussion.

5.3 Renal Effects of Angiotensins.

5.3.1 Urine Flow Rates.

Figure 5.1 shows the effect of perfusion pressure on urine flow rate in the isolated perfused kidney. As may be seen, urine flow rate varies directly with perfusion pressure. In the eel, *Anguilla rostrata*, there is a linear correlation between systemic blood pressure, glomerular filtration rate and urine flow rate (Rankin et al., 1967; Butler, 1969). These data simply show that the same phenomenon exists in the trout kidney as there is a linear correlation between renal perfusion pressure and urine flow rates. Furthermore an alteration in perfusion pressure will be reflected in the urine flow rate.

When angiotensin I (AI) is injected into the arterial side of the kidney vascular tree, urine flow rate shows a slight increase over the dose range as may be seen in Figure 5.2. These data imply that AI has some activity in the trout kidney and indeed AI appears to have an effect on renal blood flow in mammals (Peach, 1977). However, since this increase is not significant this implies that if AI does have some activity in the trout kidney it is only weakly active. Further evidence for AI having some activity in trout kidney may be seen in Figure 5.3. This figure shows the effect of injecting AI into the venous side of the kidney vascular tree and as in the previous case, the urine flow rate shows a slight non-significant increase.

The lack of a significant response may be due either to a lack of activity of AI on the kidney or that AI is only weakly active in the kidney. The latter hypothesis appears more likely as it is known that AI will compete, to a certain extent, with angiotensin II (AII) for the latter compound's recep-
Figure 5.1. Urine flow rates and perfusion pressure in the isolated perfused trout kidney. The y-axis represents the urine flow rates in ml urine formed per kg body weight per hour. The x-axis represents perfusion pressure and is measured in cm H$_2$O. For the line equation $y = 0.01x + (-0.02)$ the correlation coefficient $r = 0.969$ is significant at the 1% level. (N = 10.)
$y = 0.01x + 0.02$
Figure 5.2. The effect of injection of angiotensin I into the arterial side of the renal circulation on urine flows. The y-axis is urine flow rates in ml urine formed per kg body weight per hour and the x-axis is does of angiotensin I administered in ng. Each point on the graph represents the mean ± S.E.M. of 6 determinations.
Figure 5.3. Urine flow rates following angiotensin I injection into the venous side of the renal circulation. The y-axis represents the urine flow rates in ml urine formed per kg body weight per hour and the x-axis represents the dosage of angiotensin I injected in ng. Each point on the line represents the mean ± S.E.M. of 6 determinations.
tors (Needleman et al., 1972) and thus have a weak vasoconstrictive effect. In addition, Al may be converted to AII in the kidney as Haber et al. (1972) have found that there is a net conversion of AI to AII in the kidney although the majority of the AI appears to be degraded to smaller inactive peptides. These authors also found that the rate of conversion was too rapid to be accounted for by plasma enzymes alone which implies that the renal vasculature possesses the converting enzyme.

There are two possible explanations for the increase in urine flow rates, one being a vasoconstrictive effect and the other being a direct tubular effect. In the rabbit, Al injected into the isolated perfused kidney causes an increase in perfusion pressure, glomerular filtration rate and urine volume (Regoli, 1972). This was taken as evidence that Al had a vasoconstrictive effect on the efferent glomerular arteriole but not on the afferent vessel. This situation may exist in the fish kidney so that Al will cause efferent arteriolar constriction thus increasing the renal perfusion pressure and thus eventually urine flow rate. Since Al is only weakly active the change is not significant. In addition to smooth muscle, fish renal veins also contain groups of chromaffin cells (Gannon, 1972). It is known that either Al or AII can cause release of either epinephrine or norepinephrine from chromaffin tissue (Opdyke and Holcome, 1978). Therefore injection of Al into the venous circulation would result in release of epinephrine from the chromaffin tissue which in turn could result in some degree of vasoconstriction (probably very slight) leading to an increased glomerular perfusion pressure. This increased perfusion pressure would be a result of increased downstream resistance and would result in an increase in urine flow rates. Since veins are thin-walled vessels this would explain why there was no significant effect from Al. It is possible that the Al could have a direct
effect on the tubule such that water loss from the peritubular vessels increased due either to increased tubular secretion of water or increased secretion of an ion, such as sodium, and water follows passively. This possibility will be examined more closely in Section 5.3.2.

Injection of AII into the arterial system results in a massive decrease in urine flow rates which is dose-dependent (Figure 5.4). The most probable reason for this decrease is that the AII is causing severe vasoconstriction of the afferent glomerular arterioles which would result in a decreased perfusion pressure and a decreased urine flow rate. On the other hand, Nishimura and Sawyer (1976) found that infusion of AII into freshwater adapted and saline loaded eels (Anguilla rostrata) produced a diuretic effect which these authors concluded was the result of an increase in systemic blood pressure. Regoli (1972) concluded that AII has as its primary target the efferent arteriole to increase perfusion pressure and thus, urine volume. Brown et al. (1972) concluded that studies with exogenous AII were pharmacological rather than physiological in nature as it is the location of the converting enzyme activity which determines the target for AII. A similar situation may exist in fish as injection of AII into the renal venous circulation results in an increased urine flow rate (see Figure 5.5) which is apparently dose-dependent. This action may be explained as either the result of a direct vasoconstrictive effect of AII on the venous smooth muscle or by an indirect effect mediated by epinephrine or norepinephrine released from venous chromaffin cells or possibly a combination of the two factors. This would explain the relatively greater effect of AII as compared to AI. In addition, the AII may have a direct effect on the renal tubule such that ion transport is affected which in turn could cause an increase in urine flow. Another possible site of action of AII could be on the neck and intermediate
Figure 5.4. Urine flow rates following angiotensin II injection into the arterial side of the renal circulation. The y-axis represents the urine flow rates in ml urine formed per kg body weight per hour and the x-axis represents the dosage of angiotensin II administered in ng. Each point on the line represents the mean ± S.E.M. of 6 determinations.
Figure 5.5. The effect of injection of angiotensin II into the venous circulation of the kidney on urine flow rates. The y-axis represents the urine flow rate in ml urine formed per kg body weight per hour and the x-axis represents the dosage of angiotensin II administered in ng. Each point on the line represents the mean ± S.E.M. of 6 determinations.
segments of the nephron which are ciliated and may function as a means of propelling the filtrate down the tubule (Hickman and Trump, 1969). AII may cause an increase in activity in these regions thus increasing the rate at which the filtrate is propelled down the tubule and therefore the urine flow rate. However, this is speculative at this time as there is some controversy over the function of these segments.

5.3.2 Urinary Sodium Levels.

When AI is injected into the arterial and venous sides of the renal vascular tree, there is an effect on urinary sodium levels as illustrated in Figures 5.6 and 5.7. It may be seen that when AI is injected into the arterial side there is a decrease in urinary sodium levels (Figure 5.6) but this decrease is not significant at any dose level. When injected into the venous side, however, there is a significant dose-dependent decline (Figure 5.7). These data would indicate that AI has a direct effect on the nephron to increase sodium reabsorption. This effect appears to be independent of the effect on urine flow rate as AI causes an increase in this parameter (see Section 5.3.1), and in addition there does not appear to be a significant correlation between urine flow rate and urinary sodium levels. Therefore an increased urine flow rate is not due to either inhibition of sodium reabsorption or an increase in sodium secretion with water following passively.

There is little published information on the effect of AI on the renal tubules and all is concerned with mammalian kidneys. Peach (1977) has suggested that this decapeptide may be considered the first potential and most primitive message of the renin-angiotensin system as it is the initial peptide formed via the proteolytic action of renin and the data obtained in this study seem to support this hypothesis. However, since there
Figure 5.6. Urinary sodium levels following injection of angiotensin I on the arterial side of the renal circulation. The y-axis represents urinary sodium levels and is in meq/l Na$^+$ and the x-axis is dosage of angiotensin I administered in ng. Each point on the line represents the mean ± S.E.M. of 6 determinations.
Figure 5.7. Urinary sodium levels following angiotensin I injection into the venous circulation of the kidney. The y-axis represents urinary sodium levels in meq/l and the x-axis represents the dosage of angiotensin I administered in ng. Each point on the line represents the mean ± S.E.M. of 6 determinations.
was no inhibition of converting enzyme activity it is possible that the AI is being converted to AII which is then exerting the observed effect.

Figure 5.8 shows the effect of AII on urinary sodium levels when the octapeptide is injected into the arterial side. There is a significant dose-related decrease in urinary sodium levels. Again this decrease is not related to the urine flow rate since there is no significant correlation between the two parameters. These data are apparently in direct opposition to the findings of Nishimura and Sawyer (1976) who observed a natriuretic response correlated with the diuretic response in AII infused eels. This difference could be a result of AII not reaching the nephron receptor in the infused eel and therefore sodium retention is not affected. These data would indicate that AII has a direct effect on the nephron to cause an increase in sodium reabsorption from the tubular fluid. This hypothesis is supported by the data obtained when AII is injected into the venous side of the renal circulation as is shown in Figure 5.9. Again there is a significant dose-related decrease in urinary sodium levels following injection of AII. This effect has also been observed in mammalian species where the injection of AII caused consistent reductions in the excretion of sodium and chloride which could not be attributed to decreases in glomerular filtration rate or renal plasma flow (Melton and Frazier, 1967; Johnson and Malvin, 1977).

One other possibility is that the injected angiotensins are causing a release of cortisol or some other corticosteroid from the interrenal tissue in the trout kidney. Cortisol is the major adrenocorticosteroid in trout interrenal tissue, while aldosterone is not present in detectable quantities (Columbo et al., 1971). The renin-angiotensin system may have little effect on cortisol release as Nishimura et al. (1976) were unable to find any correla-
Figure 5.8. Urinary sodium levels following injection of angiotensin II into the renal arterial circulation. The y-axis represents the urinary sodium levels in meq/l and the x-axis represents the dosage of angiotensin II administered in ng. Each point on the line represents the mean ± S.E.M. of 6 determinations.
URINARY SODIUM LEVEL (meq/l)
Figure 5.9. Urinary sodium levels following injection of angiotensin II into the renal venous circulation. The y-axis represents the urinary sodium levels in meq/l while the x-axis represents the dosage of angiotensin II administered in ng. Each point on the line represents the mean ± S.E.M. of six determinations.
tion between plasma renin activity and cortisol levels in either the eel or the toadfish (*Opsanus tau*) upon adaptation to dilute media. In addition, it is known that the role of AII in aldosterone release in mammals is to increase the initial step in the biosynthesis of aldosterone, i.e., the conversion of cholesterol to pregnenolone (Aguilera and Marasic, 1971), but the major biosynthetic pathway from cholesterol to cortisol proceeds via 17α-hydroxypregnenolone in fish (Sandor et al., 1966). Therefore the AII catalyzed step is apparently lacking in teleosts.

In addition the similarity between the magnitude of the response produced by Al and AII (Figures 5.7 and 5.9) on the venous side of the circulation suggest that either there is no difference in activity of the two compounds or that all of the Al is being converted to AII. The latter suggestion appears unlikely based on the evidence provided by the effect of Al and AII on the urine flow rate (Figures 5.3 and 5.5) as the response produced by the AII is approximately double that produced by the Al. This suggests that Al and AII have equal activity when concerned with sodium transport and indeed in some tissues, adrenal medulla and medullary blood vessels of mammals, the affinity of Al and AII is about equal (Peach, 1977). The physiological role of this equivalent affinity is at present obscure.

### 5.3.3 Urinary Potassium Levels

Urinary potassium levels are also affected by either Al or AII but in this case the effects are much less clear-cut. Figure 5.10 shows the effect of injection of Al in the arterial side on potassium levels and as may be seen there is a slight decrease but this is not significant and does not appear to be dose-related. However, injection of Al on the venous side produces a significant dose-dependent decline in urinary potassium (Figure 5.11). These data indicate that the Al has a direct effect on the nephron
Figure 5.10. Urinary potassium levels following injection of angiotensin I into the renal arterial circulation. The y-axis represents the urinary potassium concentrations in meq/l while the x-axis represents the dosage of angiotensin I administered in ng. Each point on the line represents the mean ± S.E.M. of six determinations.
Figure 5.11. Urinary potassium levels following angiotensin I injection into the renal venous circulation. The y-axis represents the urinary potassium concentrations in meq/l while the x-axis represents the dosage of angiotensin I administered in ng. Each point on the line represents the mean ± S.E.M. of six determinations, with the exception of the initial point where the standard error was smaller than the size of the symbol.
to increase the reabsorption of monovalent cations as it does result in an increase in sodium reabsorption as was indicated in the previous section. This would explain the apparent lack of effect of the AI on the arterial side as in this case it is possible that the hormone is simply not reaching the site of action.

AII apparently does not have an effect on urinary potassium levels for while injection of AII on the arterial side results in a decrease in urinary potassium (Figure 5.12) which is not significant, injection of AII on the venous side results in a slight rise in urinary potassium (Figure 5.13). It is possible that the angiotensin may act on the tubule to cause potassium reabsorption as it is known to do in mammals (Melton and Frazier, 1976). This does not appear likely as there is little effect on urinary potassium levels following AII administration. However, these data do show that sodium retention resulting from either AI or AII administration is not a result of sodium-potassium exchange as urinary potassium levels do not increase significantly.

The lack of effect of AII on the urinary potassium levels, however, is not surprising. Potassium is abundant in both plant and animal cells and thus relatively large quantities of the ion are obtained in the diet. In addition, there is very little potassium, when compared to sodium, found in the extracellular fluid and therefore filtered potassium levels are low. The end result is that potassium loss to the environment does not pose a major problem for the organism. What is surprising is that AI should cause potassium retention at the tubular level. This may be due to activation of a non-specific transport protein in the cells of the nephron by AI which will pick up either sodium or potassium. It is also possible that this effect is
Figure 5.12. Urinary potassium levels following injection of angiotensin II into the renal arterial circulation. The y-axis represents the urinary potassium concentrations in meq/l while the x-axis represents the dosage of angiotensin II administered in ng. Each point on the line represents the mean ± S.E.M. of six determinations.
URINARY POTASSIUM LEVEL (meq/l)
Figure 5.13. Urinary potassium levels following injection of angiotensin II into the renal venous circulation. The y-axis represents the urinary potassium concentrations in meq/l while the x-axis represents the dosage of angiotensin II administered in ng. Each point on the line represents the mean ± S.E.M. of six determinations.
merely an artefact of the flame photometry although this seems unlikely when viewing the other data.

The potassium data also indicate that the diuretic effect of AII (Section 5.3.1) is a result of the vasoconstrictor action. Potassium levels do not change significantly despite the increased urine flow rates and this shows that the flow rate increase is not a result of either decreased water reabsorption or increased water secretion. Rather it is due to an increase in G.F.R. which is most likely brought about by an increased filtration pressure due to post-glomerular vasoconstriction.

5.3.4 Summary.

Therefore, in summary, both AI and AII have a diuretic effect although the diuretic effect of AII depends on which side of the vascular tree AII is acting and the diuretic effect of AI is insignificant. Both hormones cause sodium retention and this appears to be a direct effect on the nephron. This probably occurs either in the distal segment or the collecting tubule both of which are sites of sodium reabsorption (Hickman and Trump, 1969). These data also indicate that in fish AI and AII are equally effective as an antinatriuretic hormone. These data, as well, indicate that AI has an effect on potassium excretion but that AII does not.

In conclusion, the hormones, either AI or AII, are intimately involved in water and ion regulation in fish. The data which support this hypothesis are the antinatriuretic effect of both AI and AII and the diuretic and anti-diuretic effect of AII. Also either AI or AII may act as local hormones, i.e., without a systemic action, as there appears to be converting enzyme activity present in the kidney. This activity may not be necessary to bring about sodium retention as AI and AII appear to have equivalent effects on the
tubule where sodium excretion is concerned. Another conclusion is that the AI is the primitive messenger of the renin-angiotensin system. This is based on the facts that AI is formed as a direct action of renin and is necessary for the formation of AII. Also the equivalent effects of AI and AII on natriuresis are additional support for this hypothesis.
Section VI General Discussion.

6.0 RENIN IN FISHES.

6.1 Renin in Freshwater Fishes.

The physiological role of the renin-angiotensin system in fishes is obscure at the present time and unfortunately the classical endocrinological approach of removing the source of the hormone and observing the resultant effects is inapplicable due to the fact that the hormone is produced in the kidney. The system plays a role in the renal handling of ions and water, as it is known to do in mammalian species (Peach, 1977). The data discussed in the previous section (Section 5) indicate that angiotensin I is an antinatriuretic hormone while angiotensin II is both a diuretic and antidiuretic as well as an antinatriuretic hormone. (See Figure 6.1 for a summary of the control and actions of renin.) Accordingly, it is possible that in freshwater fishes the renin-angiotensin system plays a role in osmoregulation both through direct effects on the renal tubules and indirect effects on the vascular supply to the kidney.

The primary problem faced by freshwater bony fishes may be found in the fact that these animals are hyperosmotic to the medium in which they live and thus are faced with both water influx and ion loss. The function of the kidney in these animals is therefore that of conserving ions and excreting water, rather than nitrogenous waste excretion as is found in mammals. Therefore, a hormone which acts to cause conservation of ions and to increase water excretion would be of great value to these animals.

It is possible that the renin-angiotensin system is acting in the following fashion in osmoregulation. A stimulus is received by the juxtaglomerular
Figure 6.1. Diagrammatic summary of renin release and the actions of the renin-angiotensin system in the trout.
Decreased Renal Perfusion Pressure.

Plasma Sodium Levels

- No Effect
  - Feedback Inhibition
    - RENIN
      - release
        - AI Formation
          - converting enzyme
            - AII Formation
              - Vasoconstriction
                - Afferent Arterioles
                  - Antidiuretic Action
                - Efferent Arterioles
                  - Diuretic Action

Alpha and Beta Adrenergic Receptor Blockers have no Effect on this Response.
(JG) cells causing release of renin. The stimulus may be a decline in plasma sodium or a decrease in the osmolarity of the plasma and may be either a direct effect on the JG cells or transmitted via the nervous system from a receptor located elsewhere in the animal. The renin then acts to form angiotensin I (AI) in the plasma. There are two alternate pathways at this point. Either the AI directly affects the tubule to increase sodium reabsorption or the AI is converted to angiotensin II (AII) which will affect both the vascular system and the tubule in such a fashion as to cause an increase in the urine flow rate and an increased sodium retention. The net effect therefore is to rid the animal of excess water while conserving ions.

This mechanism is based on the decreased urinary sodium levels obtained by injecting AI or AII into the isolated perfused kidney (Section 5.3.2). In addition, AII has been found to increase urine flow rate when injected into the venous side of the renal vascular tree (Section 5.3.1). Unfortunately the nature of the signal perceived by the JG cells is obscure. Published data indicate that a hypoosmotic plasma has no effect on renin release as seawater adapted *Tilapia mossambica* showed no increase or decrease in plasma renin activity on either acute or chronic adaptation to freshwater despite marked changes in plasma sodium levels (Malvin and Vander, 1967). In addition, perfusing isolated kidneys with hypertonic sodium perfusate appears to have no effect on renin release either stimulatory or inhibitory (Section 4.3). The renin-angiotensin system may be more important in the freshwater fishes, since Mizogami et al. (1968) have shown that marine teleosts possess less renin per unit weight of kidney tissue than freshwater teleosts. The possibility exists that marine teleost renin is more active than freshwater teleost renin and therefore less renin per unit.
weight. Kidney mass is required by marine teleosts. Chromatography studies however show that teleost angiotensins appear to have the same structure and that there is little difference between those produced in marine teleosts and those from freshwater teleosts (Nakajima et al., 1971).

Renin may be continuously released in low levels from the JG cells and a certain level of circulating hormone is required for kidney function in freshwater teleosts. The half-life of circulating renin in humans is in the order of 120 minutes (Hannon et al., 1969) and it is entirely possible that an equivalent half-life may be found in fishes. This would in turn result in a certain level of circulating AI and AII and these hormones may be the actual substances required for the maintenance of kidney function. Also, Park et al. (1978) have proposed that two pools of renin exist in the kidney of dogs, one pool releases renin at a constant low rate and the other responds to stimuli which are associated with renin release. It is entirely possible that a similar situation exists in fishes, that is one pool for constant low level release and the other for response to extraphysiological conditions.

One argument against this hypothesis is that renin release is inhibited by AII in a negative feedback loop in trout (Section 3.5.4) as well as in mammals (Michelakis, 1971). Thus continuous production of AII should inhibit further renin release from the kidney. On the other hand, Nakajima et al. (1971) have found that teleost angiotensins are very susceptible to proteases. Thus, the formed AII may be degraded before it can reach the JG cells to inhibit renin release. The feedback system may only be operative when relatively large quantities of renin are released into the circulation and AII is formed faster than it can be broken down so that some may reach the JG cells to inhibit further renin release. Unfortunately, it
is extremely difficult to determine if there is a circulating plasma level of renin as removal of blood to measure levels of the hormone causes renin release as was demonstrated in Section 3.5.1.

The renin-angiotensin system may also play a role in response to haemorrhage in teleosts. A fall in blood volume is usually accompanied by a decline in systemic blood pressure which in turn results in a decline in renal perfusion pressure. Renin is then released in response to the fall in renal perfusion pressure (Section 3.5) and the end result is the formation of AII. There are two possible actions of this hormone, one being an anti-diuretic effect and the other an antinatriuretic effect. The former action would be a result of afferent arteriolar constriction and would result in water conservation to ultimately aid in restoring plasma volume. The latter action is the result of a direct tubular effect and would be necessary for the following reason. In the trout one response to blood loss is an increase in ventilation volume (Smith, pers. comm.) which could result in an increased water uptake at the gills. While this increase aids in restoring plasma volume, the plasma is rendered hypoosmotic. Thus sodium conservation at the kidney level would aid in restoring the osmolarity of the plasma.

During periods of prolonged exercise in trout, certain physiological changes occur and these changes may, in part, be mediated by the renin-angiotensin system. Exercise is accompanied by an increase in ventilation which in turn results in an increase in both water uptake and sodium loss at the gills (Wood and Randall, 1973a). In addition, dorsal aortic blood pressure increases but this increase appears transitory. A further effect is a prolonged diuresis which begins shortly after the onset of exercise and this increase in renal water efflux is observed over the entire period of
exercise (Wood and Randall, 1973c). The decline in dorsal aortic blood pressure could result in a decline in renal perfusion pressure and thus release of renin. An increase in plasma renin levels could thus explain the prolonged diuresis as AII is known to have a diuretic effect in whole animals as a result of increased systemic blood pressure (Nishimura and Sawyer, 1976). In addition, AII has a diuretic effect on the kidney itself when present in the renal venous circulation (Section 5.3). Also, Wood and Randall (1973b) have found that over prolonged exercise sodium efflux is initially greater than sodium influx but after 2-3 hours this situation is reversed and disturbances in renal function tend to disturb the observed pattern. Since both AI and AII have an antinatriuretic effect on the kidney (Section 5.3), it is possible that angiotensin-mediated sodium conservation at the kidney is aiding in the maintenance of this balance. Thus, the renin-angiotensin system may play a role in exercise by causing water excretion and sodium conservation. It must be pointed out, however, that the diuresis may be due to the increase in dorsal aortic blood pressure and consequently filtration pressure. But the dorsal aortic blood pressure increase is transitory and therefore the diuresis may be a result of a combination of these factors.

Therefore to summarize briefly, the renin-angiotensin system probably plays a role in osmoregulation in freshwater teleosts. It is established that both AI and AII have equivalent antinatriuretic effects on trout kidney and that AII may have a diuretic effect in normal physiological conditions. These hormones may be necessary for the maintenance of kidney function in the teleost. In order to fulfil this role renin may be continuously released, in low levels, from the JG cells. Renin may also play a role in the response to haemorrhage and in recovery from exercise.
6.2 Renin in Euryhalinity.

In addition to its role in freshwater teleosts, the renin-angiotensin system may also play a significant role in the adaptation to seawater. Renin content of eel (*Anguilla japonica*) kidneys has been observed to decrease when these animals were transferred from freshwater to seawater (Sokabe et al., 1966) and this phenomenon has also been observed in a number of other euryhaline species (Sokabe et al., 1968). The decreased renin content of the kidney may indicate that the hormone is being released from the JG cells into the blood plasma but the stimulus for this release is unknown. On the other hand, the decreased renal renin content may simply be a result of decreased renin synthesis.

When a freshwater adapted euryhaline fish is transferred to seawater several changes in blood constituents, kidney function and urine constituents are observed. The main considerations here are a decrease in dorsal aortic blood pressure (Chester Jones et al., 1969), an increase in plasma sodium levels (Sokabe et al., 1973), a decrease in glomerular filtration rates (Chester Jones et al., 1969), and a decrease in urine volume (Hickman and Trump, 1969). The decreased dorsal aortic blood pressure would result in a decreased renal perfusion pressure which in turn causes renin release. The increase in circulating levels of renin would then result in an increase in the formation of AI and consequently of AII. Since AII is a vasoconstrictor this could result in afferent glomerular arteriole constriction. The blood flow to the glomeruli is now decreased and there is a fall in the glomerular filtration rate and consequently urine flow and urine volumes. The net effect therefore is to decrease water loss through the kidney and aid in combating dehydration induced by transfer to seawater. The increase in plasma
sodium may also be partly a result of the increased plasma renin activity as both angiotensins have been shown to cause a decrease in urinary sodium levels which would in turn result in a slight increase in plasma sodium.

The mechanism for the decrease in dorsal aortic blood pressure on transfer to seawater remains obscure. This may be a result of a simple initial water efflux across the gills following transfer to the hyperosmotic medium. On the other hand the decrease may be attributable to some other stimulus such as a neural reflex. In any case the drop in dorsal aortic blood pressure would definitely result in a drop in renal perfusion pressure. This decreased perfusion pressure would result in an increased renin release (see Section 3.5.2) and this action is a result of a direct effect on the JG cells rather than through some neural-humoral reflex (see Section 3.5.3). The increase in plasma renin activity resulting from this stimulus could actually remove the source of stimulation by the vasoconstrictive action of AII causing an increase in the renal perfusion pressure. However this does not appear to be the case as renal perfusion pressure has no effect on hypotension-induced renin release in the trout (see Section 3.5.2) and renin release is only inhibited by a short-loop negative feedback system involving angiotensin II (see Section 3.5.4).

The short-loop negative feedback system should inhibit further renin release and thus show only an acute reduction in glomerular filtration rates and urine flow rates. But this does not apparently occur. This could be explained by a fairly long half-life for circulating renin, or inhibition of angiotensinases or by some other mechanism. The most logical possibility is that the generated AII constricts the afferent arterioles above the level of the JG cells. This would result in a further drop in perfusion pressure being perceived by the JG cells and a further increase in the amount of
renin released by these cells. The apparent decline in renal renin content observed by Sokabe et al. (1966) indicates that some such mechanism is operating as this is a long-lasting decline, up to 11 weeks in some species.

The antinatriuretic effect of AI and AII would seemingly be of disadvantage to an animal faced with an increased influx of sodium resulting from freshwater to seawater transfer. However, this effect may not be of any significance due to the decreased glomerular filtration rate. That is, there is less ultrafiltrate formed, due to afferent arteriolar constriction, and although there is less sodium excreted the absolute amount of sodium retained by the kidney would in this case be negligible since there is so little fluid passing through the tubules. Thus, an increased sodium retention by the tubule is not going to have much effect if there is little sodium to be retained. Similarly, the diuretic effect of the angiotensins would count for little if the kidney is shut down.

Therefore the net effect of the increased plasma renin activity would be water conservation due to renal shutdown. Indirect support for this hypothesis may be found in another species (Gasterosteus aculeatus) where a decrease in renal perfusion pressure results in a decreased size of glomerulus to Bowman's capsule ratio (Bonga, 1976). This could be due to afferent arteriolar constriction caused by an increased renin release resulting in a decline in blood flow and consequent loss of blood volume of the downstream vessels. In addition, the renin-angiotensin system is known to affect other organs than the kidney in mammalian species (Oparil, 1977) and it is possible that the system may be affecting intestinal absorption of water and electrolytes during adaptation to seawater. It may also have an effect on the gill vessels under these conditions to ensure further conservation of water and excretion of undesirable ions. However, this remains purely speculative at
the present time as there is little or no published information concerning
the effects of the renin-angiotensin system on target organs other than the
kidney in the teleost fishes.

6.3 Evolution of the Renin-Angiotensin System.

The renin-angiotensin system is found throughout the vertebrate kingdom
with the exception of cyclostomes and elasmobranchs (Sokabe et al., 1969) but
the physiological role of this system has been studied extensively only in
the mammals. Renin release is known to be affected by a large number of
factors in mammals such as catecholamines, changes in renal perfusion
pressure, changes in plasma sodium and the sympathetic nervous system (Davis
and Freeman, 1976). The angiotensins also have a multiplicity of functions
in mammals ranging from acting as a local hormone at the kidney level to a
systemic vasoconstrictor (Peach, 1977). But since this hormonal system
first appeared in the primitive bony fishes (Nishimura et al., 1971) the
question that remains to be answered concerns the evolution of the system.
Hormonal systems evolve through either modifying the structure of the hormone
or modifying the receptor such that additional functions may be added or the
system may assume an entirely different role in the physiology of organisms
throughout the phylogenetic scale.

From the data gathered in this study, one may conclude that there is a
good possibility that the most primitive situation had Al as an end product of
the system. This conclusion is based on first, the antinatriuretic effect
of Al which is equivalent to that exerted by AII; second, the potassium
retaining effect of Al which is not exerted by the AII; and third, the fact
that Al is necessary for the formation of AII. Another conclusion that may
be drawn from these data is that the earliest function of the system was to
cause sodium retention, a definite advantage for animals which are evolving
in a hypoosmotic medium.

The next probable step in the evolution of these hormones can be found in the diuretic effect exerted by AII. Again this is an advantage for the primitive fishes for not only are they faced with ion loss to the environment but in addition they are faced with water influx. A diuretic hormone thus becomes quite desirable to aid the animal in ridding itself of excess water. Since the AI is not very effective as a diuretic (Section 5.3.1) this involved modifying either the receptors or the hormone and it would appear that it was the hormone which was modified, as it changed from a decapeptide to an octapeptide. This change probably promoted the vasoconstrictor action which is probably the main cause of the diuretic effect.

As the bony fishes proliferated some groups began to invade the marine environment. This imposed a new series of stresses on the animals' osmoregulatory mechanisms. Since fishes are unable to excrete a concentrated urine, then the next best way to prevent water loss through the kidney is to reduce the functional volume of this organ. While this would have severe repercussions on nitrogenous waste excretion in mammals, it would not have a similar effect in fishes as these animals excrete nitrogenous wastes in the form of ammonia across the gills. It is possible at this point in the evolutionary time scale that the antidiuretic action of AII became more prominent although this function may have evolved as a response to blood volume loss such as haemorrhage. Therefore the primitive functions of the angiotensins are to act as an antinatriuretic, a diuretic and an antidiuretic hormone.

The diuretic and antidiuretic actions of AII are probably brought about as a result of an effect via a vascular receptor. Renin is released into
the afferent glomerular arteriole and presumably AI is formed quite rapidly. 
The action of AII which is formed from this AI now depends on two factors, 
the location of converting enzyme activity and the amount of renin released. 
Low levels of plasma renin activity and converting enzyme activity in the 
efferent side of the renal circulation will result in efferent vascular con­ 
striction, a rise in filtration pressure due to increased efferent resistance 
and thus diuresis. On the other hand, high levels of plasma renin activity 
will result in afferent arteriolar constriction as large amounts of AII will 
be formed. High levels of the hormone in the blood will increase the prob­ 
ability of the hormone reaching the afferent glomerular arterioles. Here it 
would cause vasoconstriction, which decreases glomerular filtration and the 
result is antidiuresis.

It is in the mammals that the system apparently attains the greatest 
degree of complexity. It has not only a diuretic, an antidiuretic and an 
antinatriuretic action but also displays a natriuretic action, probably due 
to the vasoconstrictor action of AII (Peach, 1977). Angiotensins are also 
known to induce behavioural modifications in mammals. Intracerebral injec­ 
tions of AII induce a drinking response (Regoli et al., 1974). Such effects 
have not been investigated in other vertebrates to any great extent but 
intraperitoneal injection of AII will cause drinking responses in the common 
iguana, Iguana iguana (Fitzsimmons and Kaufman, 1977).

In addition, the hormonal system may also be linked to other hormones 
which are known to affect the kidney. AII may be responsible for the 
increased biosynthesis of aldosterone that occurs when mammals are faced with 
a hypotonic sodium plasma, this action leading to sodium retention at the 
kidney level (Peach and Chiu, 1974). On the other hand, the AII may be 
进一步 modified, to carry out this function, into a heptapeptide form known
as angiotensin III or AIII (Williams et al., 1974). While this effect has not been extensively examined in the other vertebrates, Nishimura et al. (1976) were unable to show a renin-corticosteroid axis in fish. Also, AII has been shown to result in an enhanced vasopressin release from the posterior pituitary which could partially account for the antidiuretic action of AII but the physiological significance of this interaction is doubtful as the antidiuretic response of AII may also be attributed to its vasoconstrictor action (Peters and Bonjour, 1971; Claybaugh and Share, 1972).

Thus as the renin-angiotensin system evolved, it can be seen that the angiotensins have been altered from a decapeptide, with a specific action, to an octapeptide, with much more general effects, to a heptapeptide, again with a very specific action. But the question remains, have the receptors for this system been altered?

In fishes there appear to be two types of angiotensin receptors, a renal tubular receptor and a vascular receptor. The renal tubular receptor appears to be the more primitive of the two as it will accept either AI or AII while the vascular receptor will only accept AII. In mammals, a third type of receptor has apparently evolved and this is the adrenal cortical receptor. This latter type was believed to accept only AII but more recent work has shown that it will preferentially accept the AIII form (Williams et al., 1974). Mammals have the vascular receptor and in addition may still possess the tubular receptor as AII has a direct effect on tubular sodium reabsorption in dogs (Melton and Frazier, 1976; Johnson and Malvin, 1977). But the tubular effects of the angiotensins may not be of any physiological significance in mammals as the primary sodium retention hormone is aldosterone. Thus the receptors have not appreciably changed in nature over the evolutionary tree.
The next question is what is the basic stimulus for renin release? Since the angiotensins are involved in sodium retention, it might be expected that a fall in plasma sodium or a decrease in blood osmolarity would initiate renin release. In fish there are little or no data to support this hypothesis. A fall in plasma osmolarity or a decreased electrolyte concentration oppose a reduction in renin release caused by isotonic volume expansion in lungfish (Blair-West et al., 1977). But transfer of seawater adapted teleosts to freshwater does not always produce an increase in plasma renin activity despite marked changes in plasma sodium and blood osmolarity (Malvin and Vander, 1967; Nishimura et al., 1976). This suggests that the hypoosmotic stimulus may have existed in primitive fishes but was lost in the more modern animals. In addition perfusion of trout kidneys with a hypertonic sodium solution has no effect on renin release, which suggests that there is no ionic stimulus for renin release in teleosts. Renin release is correlated, however, with blood loss and the actual stimulus for release is a fall in renal perfusion pressure. This situation would be useful in freshwater to seawater transfer as there is a fall in dorsal aortic blood pressure in these conditions.

In mammals, renin secretion is controlled by several mechanisms and these are, the central nervous system, renal perfusion pressure and the macula densa. The latter two mechanisms appear to be independent of the central nervous system (Winer et al., 1969). The macula densa mechanism probably evolved as the renal portal system was lost since fishes, amphibians and reptiles which possess a functional renal portal system, have no macula densa. The birds, which possess a remnant of the portal system, have an intermediate type structure and the mammals, which do not have a portal system, have a fully developed macula densa (Sokabe and Ogawa, 1974). This
loss of the portal system and the evolution of the macula densa probably
came about as mammals are faced with the problem of water loss rather than
ion loss. This then requires a system whereby water may be conserved and
excess ions excreted. To fulfil this role the mammals have evolved a
kidney which is capable of producing a blood hypertonic urine and thus water
may be conserved while ridding the animal of excess ions and nitrogenous
wastes. The structure of this mechanism is such that the presence of a
portal system would preclude production of the concentrated urine. It is
possible, however, for mammals to lose ions as well and this event must be
guarded against. This is achieved through the macula densa mechanism. The
macula densa appears inhibited by high renal tubular sodium and stimulated
by low renal tubular sodium levels. Thus the renin-angiotensin system may
now respond to changes in blood volume, as reflected in changes in renal
perfusion pressure, and changes in plasma osmolarity, as reflected in changes
in renal tubular sodium load.

Therefore, it seems entirely probable that the renin-angiotensin system
evolved from a blood volume maintenance hormonal system to a much more
complex system as is evident in the mammals. Unfortunately there are several
gaps in the scheme as presented and further work must be done with this
system to ascertain exactly what role the renin-angiotensin system plays in
the physiology of the non-mammalian vertebrate species.

6.4 Summary.

1. The rainbow trout has been found to produce renin in its kidneys. This
renin forms a product similar enough to mammalian angiotensin I to allow it
to bind to a human anti-angiotensin I and thus renin activity in the trout
may be measured by a radioimmunoassay technique.
2. A decrease in renal perfusion pressure causes an increase in renin release and this increased renin release is a result of a direct effect on the J.G. cells. Renin release is not inhibited by removing the stimulus but rather by a hormonal short-loop negative feedback system involving angiotensin II.

3. Hypertonic plasma sodium levels have no direct effect on plasma renin levels in the trout as perfusion of the trout kidney with hypertonic sodium solutions has no effect on renin release either stimulatory or inhibitory.

4. Angiotensin II may be a diuretic hormone or an antidiuretic hormone depending on what part of the renal vascular tree is stimulated. Both angiotensin I and angiotensin II have a direct antinatriuretic effect on the renal tubule in the trout.

5. The physiological role of the renin-angiotensin system is obscure in freshwater teleosts but it may play a role in blood volume maintenance in response to haemorrhage and sodium retention during exercise. In euryhaline teleosts it may aid in seawater adaptation by causing renal shutdown to conserve water.


### APPENDIX 1.

Urinary Sodium Levels Following Hypertonic Saline Perfusion of the Isolated Filtering Trout Kidney.

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>Begin Perfusion. (meq/1)</th>
<th>End Perfusion. (meq/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic Saline</td>
<td>8.47 ± 1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.74 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypertonic Saline</td>
<td>12.8 ± 1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.4 ± 2.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- All values are means ± S.E.M. (N)
- <sup>b</sup> P < 0.05
- <sup>c</sup> P < 0.01

### APPENDIX 2.

Urinary Potassium Levels Following Hypertonic Saline Perfusion of the Isolated Filtering Trout Kidney.

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>Begin Perfusion. (meq/1)</th>
<th>End Perfusion. (meq/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic Saline</td>
<td>0.17 ± 0.03</td>
<td>0.2 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypertonic Saline</td>
<td>0.29 ± 0.05</td>
<td>0.38 ± 0.015&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- All values are means ± S.E.M. (N)
- <sup>b</sup> P < 0.01
Appendix 3

Figures A.1 and A.2 are sample standard curves for the bioassay and radioimmunoassay techniques respectively. The bioassay was the standard rat vasopressor assay which involved cannulation of the carotid artery and the jugular vein. The artery was used to record blood pressure while the vein was used for injection purposes. The procedure followed involved injection of a standard dose of angiotensin II, the animal was allowed to recover as evidenced by the return of the blood pressure to pre-injection levels and then a measured amount of the unknown was injected until the blood pressure showed a 5 mm Hg pressure increase. An equivalent volume of isotonic saline was injected as a control.

The mean arterial pressure was calculated by the formula: diastolic pressure plus one third of the systolic-diastolic pressure difference. The mean arterial pressure difference was calculated by subtracting the pre-injection mean arterial pressure from the post-injection mean arterial pressure.
Figure A.1. Standard curve for the rat vasopressor bioassay. The y-axis represents the dose of A-II administered, in nanograms while the x-axis represents the calculated mean arterial pressure increase.
Figure A.2. Standard curve for angiotensin I radioimmunoassay. The y-axis represents the proportion of radioactive to non-radioactive antigen (angiotensin I) bound to the antibody and is in a percentage of the total amount of radioactivity used per tube. The x-axis is the amount of non-radioactive angiotensin I and is in nanograms.
Appendix 4

List of Abbreviations

AI  Angiotensin I
AII Angiotensin II
AIII Angiotensin III
BA  Bioassay
CNS Central Nervous System
DOC Desoxycorticosterone
EDTA Ethylenediaminetetraacetic acid
JG  Juxtaglomerular
JGA Juxtaglomerular Apparatus
JGC Juxtaglomerular Cell
MD  Macula Densa
PRA Plasma Renin Activity
PVP Polyvinylpyrrolidinone
RG  Renin Granules
RIA Radioimmunoassay
SEM Scanning Electron Microscope.