

PLASMA CALCIUM AND BLOOD PRESSURE
IN THE AMERICAN EEL, ANGUILLA ROSTRATA LESUEUR,
WITH PARTICULAR EMPHASIS ON THE ROLE OF
THE CORPUSCLES OF STANNIUS

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ABSTRACT

The Corpuscles of Stannius are oval, ductless glands located on or in the substance of the mesonephric kidney of teleost fishes. Surgical removal of these glands from tap-water adapted eels (Anguilla spp.) resulted in hypercalcemia and hypotension. The present study was undertaken to determine if the hypotensive response to Stanniectomy was purely a consequence of disturbed calcium homeostasis.

It was found that Stanniectomized eels in an acalcemic environment remained normocalcemic and normotensive. But infusion of calcium solution into intact eels produced a state of hypercalcemia and a decrease in blood pressure of the same order of magnitude as that seen in Stanniectomized eels held in tap-water, as the plasma calcium levels in the intact infused eels approached those of Stanniectomized eels. Very low levels of plasma ionic calcium were also associated with significant decreases in blood pressure. Saline extracts of the Corpuscles of Stannius did not cause a hypertensive response in intact eels. The conclusions drawn from this study were that any major deviation in plasma calcium away from normal values results in a decrease in blood pressure and that the hypotensive response following Stanniectomy is a result of the disturbed plasma calcium levels.

The effects of hyper- and hypo-calcemia on blood pressure are discussed in terms of a possible mechanism of action on intrinsic or extrinsic factors which control blood pressure either directly or indirectly.

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INTRODUCTION

PLASMA CALCIUM AND BLOOD PRESSURE IN THE AMERICAN EEL, ANGUILLA
ROSTRATA (LESUEUR) WITH PARTICULAR EMPHASIS ON THE ROLE
THE CORPUSCLES OF STANNIUS

1. Histological Appearance and Early History

The Corpuscles of Stannius appear as oval, ductless glands and are found on or in the mesonephric kidney of teleost fishes (Stannius, 1839). Embryologically, they develop as protrusions from the pronephric uriniferous tubule (Huot, 1898; Giacomini, 1929-34; Garrett, 1942, de Smet, 1962). Recently, Krishnamurthy (1967) reported that differentiation into Corpuscles of Stannius had also taken place in the mesonephric uriniferous tubules of Colisa lalia. There is usually one pair of these glands in recent teleost fishes like the eels (Bauchot, 1953). But there are between 20 to 25 pairs in primitive forms like Amia calva and from 3 to 7 pairs in fishes of Oncorhynchus sp. (Garrett, 1942; Bauchot, 1953).

Macroscopically, in Anguilla rostrata, the corpuscles appear as whitish, oval bodies which are usually two or three millimeters in diameter. These glands are most frequently found at the point of fusion of the posterior kidneys. But they may be located more caudally in some cases. The blood supply appears to arise from the renal blood vessels (Chester Jones et al, 1969).

The Corpuscles of Stannius are richly innervated. The presence of ganglia and individual neurons suggests that this

innervation is of autonomic origin but it is not known whether these are sympathetic or parasympathetic fibres (Krishnamurthy and Bern, 1971).

Histologically there appear to be four types of corpuscles (Krishnamurthy and Bern, 1969):

Type I : composed of compact lobules with a single layer of cells along a connective tissue septum. This arrangement gives these lobules a tubular appearance; the lumen of these tubules is usually filled with connective tissue.

Type II : the connective tissue seems to penetrate the lobules and depending on the type of section, appears as islands or long septa scattered among the corpuscular cells.

Type III : the penetrating connective tissue joins to cause some groups of cells to become separated from the rest, forming smaller lobules from the incomplete lobes. The incomplete lobes were formed by the connective tissue and are also seen in type II. Those lobules formed are only seen in small numbers.

Type IV : the corpuscle is composed of aggregates of small lobes each of which consists of a number of incomplete lobules, Each lobe thus resembles a complete corpuscle of type II.

The Corpuscles of Stannius of the eel appear to be type I in structure. In addition, studies of the fine structure of the Corpuscles of Stannius of the Japanese eel, Anguilla japonica, have shown that the lobules are well vascularized; blood capillaries are found in the connective tissue surrounding the lobules (Fujita and Honma, 1967).

The cells of the corpuscles are characterized by a number of round or ovoid cytoplasmic granules. These granules appear to be proteinaceous in nature in both the European eel, Anguilla anguilla and the Japanese eel, Anguilla japonica (Hanke and Chester Jones, 1966; Fujita and Honma, 1967). A well developed rough-surfaced endoplasmic reticulum is also present in the corpuscular cells of both species (op.cit., 1966; op.cit., 1967). In overall appearance these cells resemble those of the exocrine pancreas (Palade, 1956). The fine structure of the Corpuscles of Stannius of the North American eel, Anguilla rostrata L., appears to be similar to that of the Japanese eel (Butler, 1969).

Earlier, because of the location of these glands, they were confused with the adrenal cortex of higher vertebrates; a situation which persisted for several years (Vincent, 1898).

The first role implied for the Corpuscles of Stannius was in hydro-mineral regulation (Rasquin, 1956). Injections of potassium chloride or prolonged immersion of the teleost Astyanax mexicanus in one per cent sodium chloride solution produced a degranulation, a loss of cytoplasm and a decrease in the size of

the corpuscular cells. Hypertrophy of the corpuscular cells resulted from administration of water or diluted pitressin. These findings all indicated that the corpuscles played a role in osmoregulation (Rasquin, 1956). It was also observed that the surgical removal of the Corpuscles of Stannius resulted in a decrease in plasma sodium and chloride in European eels. These changes could be reversed by aldosterone (Fontaine, 1964; Leloup and Leloup-Hatey, 1964; Leloup-Hatey, 1964 a & b). Due to this evidence, and the location of the corpuscles, it was postulated that the Corpuscles of Stannius were homologous to the adrenal cortex of higher vertebrates (Leloup-Hatey, 1964 a & b).

2. CORPUSCLES OF STANNIUS AND INTERRENAL TISSUE

Since later work has raised much controversy regarding the homologous nature of the Corpuscles of Stannius and interrenal tissue, the two glands will be compared. The comparisons will be made on the basis of embryological development, cellular fine structure, role in steroidogenesis and effect on steroid hormone levels in the blood following surgical removal of the Corpuscles of Stannius or the interrenal glands.

A) EMBRYOLOGY AND GROSS ANATOMY

The cells of the interrenal tissue (suprarenal tissue, adrenocortical tissue) arise embryologically from the mesodermal blastema (Chester Jones et al, 1969). The Corpuscles of Stannius on the other hand appear to arise as protrusions from either the

pronephric duct (Garrett, 1942) or the mesonephric duct (Krishnamurthy, 1967).

In amniota the interrenal tissue is organized into discrete encapsulated bodies opposed to the kidneys but in anamniota the interrenal tissue is not a separate encapsulated gland but remains closely associated with the mesonephric kidney (Chester Jones et al, 1969). In the eel, the interrenal tissue is found in the head kidney and embedded within the walls of the cardinal vein (Chester Jones et al, 1966, Chester Jones et al, 1969). This is in direct contrast to the Corpuscles of Stannius which are discrete, encapsulated bodies lying on or in the mesonephric kidney.

B) CELLULAR FINE STRUCTURE

I. CORPUSCLES OF STANNIUS*

The cells of the Corpuscles of Stannius appear as columnar secretory cells with many rounded or ovoid granules which are toluidine-blue positive. The nucleus is either elongated or ovoid and lies near the base of the cell and is parallel to the long axis of the cell.

The nucleus of the corpuscular cell possesses a distinct nucleus and a diffusely distributed chromatin. The cytoplasm appears compact and is characterized by a well developed rough-surfaced endoplasmic reticulum with a well developed Golgi area. The granules found in the cytoplasm are regular in shape and are enclosed by limiting membranes. The limiting membrane is derived from the Golgi vacuoles. The granules themselves appear to be

* Fujita and Honma (1967).

proteinaceous and are osmiophilic. Mitochondria with laminar crests are found in the cytoplasm.

II. INTERRENAL TISSUE*

The cells of the interrenal tissue appear as columnar cells with large oval nuclei or polyhedral cells with dense nuclei and the cytoplasm is characterized by numerous mitochondria. These mitochondria possess tubular crests. Many granules are found in the cytoplasm of these cells; these granules are irregular in shape, osmiophobic, lipid in nature and give the cytoplasm a foamy appearance. The cytoplasm is also characterized by the presence of a well developed smooth-surfaced endoplasmic reticulum. In light microscopy these cells are sudanophilic and contain ascorbic acid (Chavin, 1965).

From a comparison of the fine structures of the cells of these two glands, it is clear that the cells of the Corpuscles of Stannius differ markedly from interrenal cells characteristic of teleosts. While the corpuscles appear to secrete a substance which is proteinaceous in nature, the interrenal tissue is known to secrete steroid hormones (Mayer and Maetz, 1967; Sandor et al, 1967; Hirano and Utida, 1968).

C) ROLE IN STEROIDOGENESIS

It has been found that plasma cholesterol is converted to adrenocortical steroids in the eel (Sandor et al, 1966). Two

* Rhodin, 1963.

pathways appear to exist: a) from plasma cholesterol through to 17 α -hydroxypregnenolone and 17 α -hydroxyprogesterone to cortisol and cortisone (op. cit., 1966); b) from plasma cholesterol to pregnenolone through to progesterone and 17 α -hydroxyprogesterone to cortisol and cortisone. The major adrenocortical hormone produced by the eel has been shown to be cortisol although some cortisone is produced (Chester Jones and Phillips, 1960; Bern, 1965; Chester Jones et al, 1965b). It is not known which pathway is the most important but evidence suggests that pathway A is the major source of adrenocortical hormones and pathway B serves as a secondary route (Sandor et al, 1966).

Attempts have been made to extract adrenocortical-type steroids from both the Corpuscles of Stannius and the interrenal tissue of various fishes. The Corpuscles of Stannius of the Atlantic salmon, Salmo salar, possess steroids since both cortisol and corticosterone have been extracted from them (Fontaine and Leloup-Hatey, 1959). However, C₂₁ type steroids were not detected in methanol-preserved Corpuscles of Stannius of sockeye salmon, Oncorhynchus nerka (Ford, 1959). The Corpuscles of Stannius of Salmo gairdnerii have been reported to possess 11-deoxycorticosterone and 5 α -pregnenedione (Columbo, Bern and Pieprzyk, 1971). The interrenal tissue has been found, by several authors, to contain cortisol and cortisone with cortisol being present in the largest amount (Chester Jones and Phillips, 1960; Butler, 1965; Chester Jones et al, 1965; Sandor, 1966).

Incubation studies using corpuscular tissue and interrenal tissue have given confusing results. It was reported that the Corpuscles of Stannius of the cod, Gadus morhua, showed limited conversion of labelled progesterone to 11-deoxycorticosterone (Idler and Freeman, 1966). The trout, Salmo gairdnerii, corpuscles have been reported to convert labelled progesterone to 11-deoxycorticosterone and 5 α -pregnenedione (Columbo, Bern and Pieprzyk, 1971) but the data is rather ambiguous. However homogenates of the interrenal tissue from the eel, Anguilla anguilla, do not show conversion of cholesterol to cortisol. No corticosterone, aldosterone or 18-hydroxycorticosterone could be detected (Butler, 1965; Sandor, 1966). The Corpuscles of Stannius of the winter flounder, Pseudopleuronectes americanus, were unable to convert progesterone to adrenocortical steroids in vitro (Phillips and Mulrow, 1959). It appears that the Corpuscles of Stannius of the European eel, Anguilla anguilla, are unable to convert cholesterol to adrenocortical steroids although some modification of pre-existing steroids has been observed to take place (Chester Jones et al, 1965; Brewer and Ozon, 1965).

Histochemical studies have been done on both the corpuscular tissue and the interrenal tissue. The object of these studies was to demonstrate the presence of the enzyme steroid-3 β -ol-dehydrogenase which catalyzes the conversion of pregnenolone to progesterone. This conversion is one of the first steps in the biosynthesis of the adrenocortical hormones. Consistently negative

results (i.e. no steroid-3 β -ol-dehydrogenase) have been reported for the corpuscles of the European eel and those of the marine eel, Conger conger. On the other hand, head kidney with the contained interrenal tissue gave consistently positive results (Chieffi and Botte, 1963). Further studies on the European eel corroborated these results; that is, no steroid-3 β -ol-dehydrogenase could be detected in the cells of the Corpuscles of Stannius. But this enzyme was consistently found in the cells of the interrenal tissue (Chester Jones et al, 1965; Hanke and Chester Jones, 1966). It has been observed that some other enzymes in the adrenocortical steroid pathway, these being 3 α -, 11 β -, and 17 β -hydroxysteroid dehydrogenases, conform to the same pattern of distribution and are present in the cells of the interrenal tissue but are not found in the cells of the Corpuscles of Stannius (Bara, 1968).

D) EFFECT ON ADRENOCORTICAL HORMONE LEVELS

The major adrenocortical hormone in the eel is cortisol (Chester Jones and Phillips, 1960; Butler, 1965; Chester Jones et al, 1965; Sandor, 1966). Therefore, when discussing the effects of interrenalectomy and Stanniectomy on the adrenocortical hormones, only cortisol will be mentioned. The effects of hypophysectomy on the interrenal gland and the Corpuscles of Stannius will also be discussed.

During the first two weeks after Stanniectomy, an increase in interrenal activity has been observed with lasting hypertrophy of the interrenal tissue (Leloup-Hatey, 1970b), but

plasma cortisol levels fell after this period (op.cit., 1970b).

It has also been found that this decrease persists for up to six weeks following Stanniectomy (Fenwick and Forster, 1972). Despite the interrenal hypertrophy, it appears that there is a decline in the amount of stable cortisol present in the blood plasma.

It may be assumed that surgical removal of the interrenal gland results in the disappearance of endogenous cortisol in the blood since significant alterations in body water, plasma electrolytes and muscle electrolytes are observed (Chester Jones et al, 1969). However, there have been no attempts to detect cortisol in the plasma of interrenalectomized eels. Attempts have been made to induce interrenal insufficiency by chemical means; these being administration of metapirone with simultaneous administration of β - or dexamethasone. These drugs result in significant changes in plasma sodium levels, muscle water and muscle sodium concentrations (Chester Jones et al, 1969). However, the secretory patterns of the adrenal cortex have not been studied. Interrenalectomy results in a reduction of the nuclear size of the cells of the Corpuscles of Stannius in fresh-water adapted eels but causes an increase in both nuclear and cellular size of the Corpuscles of Stannius in salt-water adapted eels (Hanke and Chester Jones, 1966; Hanke, Bergerhoff and Chan, 1967).

There is little evidence to indicate the presence of a pituitary trophic hormone which acts on the Corpuscles of Stannius. In the goldfish, Carassius auratus, hypophysectomy has no effect on

the histology of the corpuscles (Chavin, 1956). But hypophysectomy of the European eel results in hypertrophy of the corpuscles (Olivereau and Fontaine, 1965). Administration of ACTH has also been shown to result in hypertrophy of the corpuscles in both fresh-water and sea-water adapted eels (Hanke et al, 1967).

The effect of hypophysectomy on plasma cortisol levels has been well documented in Anguilla anguilla, A. japonica and A. rostrata. Hypophysectomy decreased circulating plasma cortisol levels and thus was associated with a marked atrophy of the interrenal tissue (Butler, Donaldson and Clarke, 1969; Chester Jones et al, 1969; Mirano, 1969; Ball et al, 1971; Fenwick and Forster, 1972; Butler, personal communication).

The conclusion which may be drawn from this comparison is that the Corpuscles of Stannius are not primarily steroidogenic tissue. These glands cannot convert cholesterol to steroid hormones because they lack the enzyme steroid-3 β -ol-dehydrogenase which converts pregnenolone to progesterone (Chieffi and Botte, 1963; Chester Jones et al, 1965; Hanke and Chester Jones, 1966). As well, the corpuscles lack various other enzymes in the adrenocortical hormone pathway (Bara, 1968). The cellular fine structure of the Corpuscles of Stannius (Fujita and Honma, 1967) differs from the cellular fine structure of the interrenal tissue (Rhodin, 1963). However, it is a possibility that the Corpuscles of Stannius may store and convert pre-existing steroids (Nandi, 1967). It has also been suggested that the Corpuscles of Stannius may function in

co-operative steroidogenesis in a manner which is analogous to the mammalian foeto-placental unit (op.cit., 1967) although there is little evidence to support this rather speculative suggestion.

3. OSMOTIC AND IONIC SEQUELAE OF STANNIECTOMY AND INTERRENALECTOMY

In this section the effects of Stannietomy and interrenalectomy on plasma, muscle and urine electrolytes will be discussed and compared. This will be done in four sections, the effects of Stannietomy, the effects of interrenalectomy and a comparison of the two. The fourth section will be the conclusions which can be drawn from this comparison.

A) EFFECT OF STANNIECTOMY

Most of the known effects of the surgical removal of the Corpuscles of Stannius are summarized in Table I.

TABLE I
EFFECTS OF STANNICTOMY

AUTHOR (YEAR)	SPECIES	MEDIUM	OBSERVATIONS
BUTLER (1969)	<u>Anguilla</u> <u>rostrata</u>	Fresh-water	Increases in plasma Ca ⁺⁺ , K ⁺ but decreases in plasma Na ⁺ , Cl ⁻ and Mg ⁺⁺ . Increases in urinary Ca ⁺⁺ and K ⁺ . Decreases in urinary Na ⁺ and Cl ⁻ . Decreases in plasma and urinary osmolalities. No significant change in G.F.R. and urine flow rates. Conclusion: corpuscles involved in ionoregulation.
(1972)	<u>Anguilla</u> <u>rostrata</u>	Fresh-water	No change in plasma metabolites such as glucose. Conclusion: corpuscles involved solely in ionoregulation.
CHAN et al (1967)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Decreases in plasma Na ⁺ and Cl ⁻ . Decreases in urinary Na ⁺ and Cl ⁻ . Serum K ⁺ increased but no effect on urine K ⁺ . Plasma calcium increases massively, approximately 100% increase.

AUTHOR (YEAR)	SPECIES	MEDIUM	OBSERVATIONS
CHAN et al (1967)	<u>Anguilla</u> <u>anguilla</u>	Salt-water	In yellow eels increases in plasma Na+, Ca++, and Mg++. No effect on K+. In silver eels same effects plus plasma Cl- and K+ increased. Effects similar to interrenalectomy.
CHAN, RANKIN AND CHESTER-JONES (1969)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Decreases in urinary Ca++, Mg++ and Na+. Decreases in systemic blood pressure and plasma Na+. Increases in plasma Ca++ and Mg++. No effect on urine volume and total body water. Conclusions: corpuscles affecting renal tubular secretion.
CHESTER-JONES et al (1965)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Decrease in blood pressure and changes in electrolytes. Possibility that corpuscles influence renal function in eels.

AUTHOR	SPECIES	MEDIUM	OBSERVATIONS
CHESTER-JONES and HENDERSON (1965)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Removal of the corpuscles does not affect Na ⁺ and Cl ⁻ uptake by the gills.
CHESTER-JONES et al (1966)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Removal of the Corpuscles of Stannius followed by a drop in blood pressure to levels which are normally found in eels adapted to sea-water. Conclusions drawn: the corpuscles of Stannius possess a pressor agent probably reinin since quite similar.
CHESTER-JONES, CHAN HANDERSON AND BALL (1969)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Removal followed by decline in plasma Na ⁺ , increase in plasma Ca ⁺⁺ and K ⁺ . Muscle water increases but extra-cellular fluid space decreases. Muscle Na ⁺ decreased while muscle Ca ⁺⁺ increased.

AUTHOR	SPECIES	MEDIUM	OBSERVATIONS
		Sea-water	Removal causes increases in plasma Na ⁺ , K ⁺ , Ca ⁺⁺ and Mg ⁺⁺ . Tissue electrolytes increase.
			In both fresh-water and sea-water increases in plasma calcium and muscle calcium transient phenomena, levels return to pre-operative in about six weeks.
FENWICK and FORSTER (1972)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Observed hypercalcemia and hyponatremia after two weeks and significantly elevated plasma cortisol levels at ten days.
		Sea-water	Observed hypercalcemia at ten days but no effect on plasma sodium. Also found a non-significant increase in plasma cortisol levels after two weeks.

AUTHOR	SPECIES	MEDIUM	OBSERVATIONS
FONTAINE (1964)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Observed plasma hypercalcemia, hyperkalemia and hyponatremia. Changes were corrected by injection of an extract of the Corpuscles of Stannius. Concluded that the corpuscles were involved in osmo-regulation in the eel.
FONTAINE (1967)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Observed decreases in plasma phosphate, sodium and chloride. Increase in plasma calcium. Changes reached peak at four weeks post-operative time but at eight weeks post-operative time electrolyte concentrations were returning to pre-operative levels.
FONTAINE et al (1972)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Calcium uptake from environment vastly increased following Stanniectomy. Data suggest that corpuscles inhibit calcium uptake across gills.

AUTHOR	SPECIES	MEDIUM	OBSERVATIONS
LELOUP and LELOUP - HATEY (1964)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Stanniectomy results in stimulation of the thyroid. Stimulation can be corrected by aldosterone. Concluded that disturbances in sodium/potassium ratio probably the cause.
LELOUP-HATEY (1964a)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Observed a decrease in plasma sodium levels and muscle sodium. An increase in plasma potassium was also observed. All found at four weeks post-operative time.
(1964b)	<u>Anguilla</u> <u>Anguilla</u>	Fresh-water	These changes in plasma sodium and plasma potassium observed after Stanniectomy were all corrected by large doses of aldosterone. Concluded corpuscles part of adrenal cortical system
LELOUP-HATEY (1970)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Stanniectomy results in lasting hypertrophy of interrenal tissue in first two weeks post operative time. Cortisol levels fall when plasma calcium reaches peak probably because high calcium levels tend to inhibit cortisol biosynthesis.

AUTHOR (YEAR)	SPECIES	MEDIUM	OBSERVATIONS
OLIVEREAU (1961)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Corpuscles of Stannius play a role in ionic and osmotic regulation especially during disequilibrium which is observed during adaptation to sea-water.
PANG (1971)	<u>Fundulus</u> <u>heteroclitus</u>	Fresh-water	Increase in total plasma calcium of approx. 300%. Serum phosphate decreases by about 50%. Hyperchloremia, hyperkalemia, and hyponatremia also observed. Stanniectomized fish showed asthenia, equilibrium loss, decreased opercular activities and calcerous concentrations in the kidney.
RASQUIN (1956)	<u>Asatyanax</u> <u>mexicanus</u>	1% saline	Corpuscles involved in osmotic regulation.
VINCENT (1898)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	At this time it was thought that the corpuscles were homologous with the adrenal cortex in mammals. Since the

AUTHOR	(YEAR)	SPECIES	MEDIUM	OBSERVATIONS
				fish lived for a long time after the operation, it was concluded that the adrenal cortex was not necessary for life.

B) EFFECT OF INTERRENALECTOMY

The major effects of interrenalectomy are presented in Table II.

TABLE II
EFFECTS OF INTERRENALECTOMY

AUTHOR	(YEAR)	SPECIES	MEDIUM	OBSERVATIONS
CHAN, CHESTER-JONES, HENDERSON and RANKIN. (1967)		<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Decrease in concentration of plasma sodium, calcium and magnesium. Decrease in concentration of muscle sodium, potassium, calcium and magnesium but increase in muscle water. Plasma potassium unaffected.
			Sea-water	Yellow eels: increase in concentration of plasma sodium and magnesium. Potassium unaffected. Decrease in muscle water but increase in muscle potassium.
				Silver eels: increased concentrations of plasma sodium, calcium and magnesium. Decrease in muscle water but increase in muscle magnesium.
			Distilled-water	Interrenalectomized eels cannot survive in distilled water.

AUTHOR	(YEAR)	SPECIES	MEDIUM	OBSERVATIONS
CHAN, RANKIN and	CHESTER-JONES (1969)	<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Decrease in glomerular filtration rate, urine volume and extra-renal uptake of sodium. Results in increased body weight, decrease in plasma sodium and magnesium and hydration of muscle cells. Changes rectified by physiological doses of cortisol and to a lesser extent 11-deoxycortisol. Aldosterone has no effect in comparable doses.
CHESTER-JONES et al (1966)		<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Yellow eels - decrease in plasma sodium and magnesium. Silver eels - decrease in plasma calcium as well. Also an increase in cellular water.
			Sea-water	Increase in plasma sodium, calcium and magnesium. Loss of water from cells and a decrease in body weight probably due to dehydration.

AUTHOR	(YEAR)	SPECIES	MEDIUM	OBSERVATIONS
CHESTER-JONES, CHAN HENDERSON AND BALL (1969)		<u>Anguilla</u> <u>anguilla</u>	Fresh-water	Decrease in plasma sodium, calcium, magnesium and chloride. Marked haemodilution and a marked rise in muscle water content. Sodium-potassium plasma ratio increased as did body weight. Muscle sodium, calcium and magnesium content decreased.
			Sea-water	Decrease in body weight which is associated with dehydration. Plasma sodium, potassium, calcium, magnesium and chloride concentrations markedly increased. Decrease in muscle water content while muscle sodium and magnesium content increase. Poor survival in full strength sea-water. but can be maintained in one-third sea-water.
			Distilled-water	Very poor survival. Interrenalectomized eels cannot live when environmental sodium falls below sixty micro-equivalents per liter.

C) STANNIECTOMY COMPARED WITH INTERRENALECTOMY

From the preceding tables it may be seen that Stanniectomy of fresh-water adapted eels results in plasma hyponatremia, hyperkalemia, hypercalcemia, an increase in magnesium levels, a decrease in plasma phosphate levels and a decrease in plasma chloride. Interrenalectomy of fresh-water adapted eels results in plasma hyponatremia, hypocalcemia, a decrease in plasma magnesium levels and a decrease in plasma chloride levels. Thus the effects of Stanniectomy and interrenalectomy on plasma electrolytes are quite similar with the exception of the calcium response. Whereas Stanniectomy results in plasma hypercalcemia, interrenalectomy results in plasma hypocalcemia.

On the other hand, the effects on urine electrolytes are markedly dissimilar. Stanniectomy of fresh-water adapted eels results in decreases in urine sodium, chloride, calcium and magnesium. Interrenalectomy results in an increase in the urine concentrations of sodium, chloride, calcium and magnesium. This seems to indicate that the interrenal tissue is influencing renal tubular reabsorption of electrolytes.

Following Stanniectomy of fresh-water adapted eels increases in muscle water and muscle calcium were observed. Blood pressure was observed to decrease, muscle sodium levels fell and the extra-cellular fluid volume decreased. No effect on glomerular filtration rate was observed (Butler, 1969). Interrenalectomy was followed by decreases in muscle sodium, calcium, magnesium and potassium. Muscle water increased but glomerular filtration rate

and urine volumes decreased. Blood pressures have not been recorded in interrenalectomized eels but adrenal insufficiency is known to result in a decrease in blood pressure in mammals (Friedman, 1965).

The effects of Stanniectomy and interrenalectomy are remarkably similar with the exception of the hypercalcemic effect of Stanniectomy. Interrenal hypertrophy is observed following Stanniectomy (Leloup-Hatey, 1970b; Fenwick and Forster, 1972) of fresh-water adapted eels. However interrenalectomy results in corpuscular atrophy (Hanke and Chester Jones, 1966; Hanke, Bergerhoff and Chan, 1967) of fresh-water adapted eels. This would suggest that the Corpuscles of Stannius respond to a hypercalcemic stimulus rather than to hyponatremia. It has been found that interrenal tissue is stimulated by increases in plasma calcium in the dog, rat and eel (Marotta and Lau, 1970; Farese, 1971 a,b; Leloup-Hatey, 1971 a,b; Milligan and Kraicer, 1971) which could explain the interrenal hypertrophy following surgical removal of the Corpuscles of Stannius.

Interrenalectomy (Table II) of sea-water adapted eels results in decreases in muscle water and body weight. Plasma hypercalcemia, hypernatremia, hyperkalemia, an increase in plasma chloride levels and an increase in plasma magnesium levels are observed. Muscle electrolytes are also observed to increase. The effects of Stanniectomy (Table I) on sea-water adapted eels are similar to the effects of interrenalectomy. But interrenalectomy of sea-water adapted eels results in more pronounced changes than

does Stanniectomy of sea-water adapted eels (Chan et al, 1967).

Corpuscular hypertrophy is observed in sea-water adapted interrenalectomized eels (Hanke, Bergerhoff and Chan, 1967). The effect of Stanniectomy on the interrenal tissue of sea-water adapted eels appears to be masked by the trauma which is a result of wounding the fish (Fenwick and Forster, 1972).

D) CONCLUSIONS TO BE DRAWN FROM COMPARISON

It has been demonstrated that the effects of Stanniectomy and interrenalectomy in fresh-water adapted eels and the same operations in sea-water adapted eels are remarkably similar. The exception to this is the hypercalcemic response obtained in Stanniectomized fresh-water adapted eels which is not seen in fresh-water adapted interrenalectomized eels. It would appear that the hypercalcemia resulting from Stanniectomy is causing inhibition of cortisol biosynthesis in the interrenal tissue (Leloup-Hatey, 1970 a,b; Fenwick and Forster, 1972).

It has been found that Stanniectomy has no effect on glomerular filtration rate (Butler, 1969), but interrenalectomy causes a decrease in glomerular filtration rate (Chan, Rankin and Chester Jones, 1969) in fresh-water adapted eels. However, since the hypercalcemic effect of Stanniectomy is transitory, and as Butler did not study renal function during the first three weeks following Stanniectomy, it has been suggested that he investigated animals returning to calcium homeostasis (Fenwick and Forster, 1972).

The weight of the evidence thus appears to favour the hypothesis that the hypercalcemia following Stanniectomy results in an inhibition of the interrenal tissue leading to adrenocortical insufficiency. This differs from the effect of hypophysectomy since it has been found that high adrenocorticotrophic hormone levels will overcome the calcium inhibition effect (Leloup-Hatey, 1970a). This would explain why the effects of interrenalectomy are more severe than those of Stanniectomy in sea-water adapted eels (Chan et al, 1967).

4. THE CORPUSCLES OF STANNIUS, THE RENIN-ANGIOTENSIN SYSTEM AND BLOOD PRESSURE

The blood pressure of fresh-water adapted eels is high relative to salt-water adapted eels; in sea-water adapted eels the blood pressure is low relative to fresh-water adapted eels (Chester Jones, Chan and Rankin, 1969). Following Stanniectomy of fresh-water adapted eels, the blood pressure drops to levels characteristic of sea-water adapted eels (Chester Jones et al, 1965a; Chester Jones, Henderson, Chan and Rankin, 1966; Chester Jones et al, 1966). It has also been shown that extracts of the Corpuscles of Stannius appear to possess a potent pressor substance which acts in both the rat and the eel (Chester Jones et al, 1966; Chester Jones, Chan and Rankin, 1969). These extracts of the Corpuscles of Stannius appeared to resemble mammalian renin in being non-diffusible through cellophane, heat-labile and destroyed by acidification to pH 2.0 (Chester Jones et al, 1966). However, it differed from mammalian renin in that the

effect in the rat was more prolonged and frequently biphasic (op.cit., 1966).

The source of renin in vertebrates will be examined and the possible role of the Corpuscles of Stannius in blood pressure will be discussed.

A) THE JUXTAGLOMERULAR APPARATUS

The juxtaglomerular cells of the kidney have been identified as the source of renin in the mammal (Bing et al, 1967; Cook, 1968). It has been shown that the juxtaglomerular cells are found in mammals, birds, reptiles, amphibians, and bony fishes but are absent in cartilaginous fishes and jawless fishes (Sokabe et al, 1969, Nishimura et al, 1970).

The juxtaglomerular apparatus consists of two types*: Type I which consists of the juxtaglomerular cells (J.G. cells), the afferent and efferent arterioles, the macula densa and the Polkissen or colour cushion. This is the type which is found in mammals.

Type II which is found in birds, reptiles, amphibians and bony fishes. This consists of the J.G. cells, the afferent arterioles and the efferent arterioles only. It has been shown that the glomerular kidney of marine teleosts has renin activity and possesses cells which are analogous to the J.G. cells of glomerular kidneys (Sokabe et al, 1969). Renin appears to be lacking in the

* Sokabe et al, (1969)

kidney of elasmobranchs and cyclostomes (Nishimura et al, 1970) and no angiotensinogen, the precursor of angiotensin I, can be found in the blood plasma of these fishes (Nishimura et al, 1970).

The J.G. apparatus (renin-angiotensin system) appears to be involved in both the maintenance of blood pressure (Turner, 1966) and the control of sodium homeostasis (Davis et al, 1967; Vander and Luciano, 1967; Aguilera and Marusic, 1971; Michelakis, 1971a; Waugh, 1972). The evidence for the role of the renin-angiotensin system in the maintenance of blood pressure is considerable, renin release being stimulated by the sympathetic nervous system, catecholamines, decreased blood pressure and decreased renal perfusion pressure (Michelakis, 1971b). It has also been postulated that stretch plays a role in the rate of renin release from the J.G. cells (Barajas, 1971).

Evidence indicates that an increase in plasma angiotensin II levels results in an increased rate of conversion of corticosterone to aldosterone (Aguillera and Marusic, 1971). This has led to the hypothesis that the increased rate of conversion of corticosterone to aldosterone in sodium-deficient animals is regulated by the renin-angiotensin system (op.cit., 1971). It has also been shown that plasma hypernatremia results in a decreased rate of renin release from the J.G. cells (Michelakis, 1971a). In dogs, when extra-cellular calcium concentrations are greater than 2.5 mM/liter a decreased renin release results (Michelakis, 1971a) but lack of calcium will result in an inhibition of renin release from the kidney (op.cit.,

1971a). Angiotensin II, by direct renal effects, may act in the regulation of salt homeostasis through renal conservation of electrolytes (Waugh, 1972).

The control of renin release remains obscure at the present time. Anatomical and histological evidence suggest that stretch may play a role in the control of renin release (Barajas and Latta, 1971). A negative-feedback mechanism has also been postulated since it was demonstrated that angiotensin II inhibits renin production and release, thus indicating a short-loop negative-feedback type of control (Michelakis, 1971b). Sodium levels in the blood plasma may also play a role in the control of renin release (Michelakis, 1971a).

B) THE CORPUSCLES OF STANNIUS AND BLOOD PRESSURE

The Corpuscles of Stannius were postulated to be part of the renin-angiotensin system (Chester Jones et al, 1966) but further work revealed that there were serious histological differences between the Corpuscles of Stannius and the juxtaglomerular cells (Capréol and Sutherland, 1968; Krishnamurthy and Bern, 1969). Also, biochemical differences between the secretions of the corpuscles and the juxtaglomerular cells were noted (Chester Jones et al, 1966; Nakajima, Nakayama and Sokabe, 1971) and differences in response to hypercalcemia and hyponatremia (Chester Jones et al, 1969; Michelakis, 1971a).

In spite of the apparent absence of an important renin-angiotensin involvement, the Corpuscles of Stannius have been presumed to be involved in the regulation of blood pressure. Surgical

removal of these glands results in a decrease in systemic blood pressure (Chester Jones et al, 1965; Chester Jones et al, 1966; Chan, Rankin and Chester Jones, 1969). Sea-water adapted eels show a decrease in blood pressure, with respect to fresh-water adapted eels (Rankin, Chan and Chester Jones, 1967; Chester Jones, Chan and Rankin, 1969). This decrease in blood pressure is accompanied by a decrease (with respect to fresh-water adapted eels) in the glomerular filtration rate (Sharratt, Chester Jones and Bellamy, 1964a; Rankin, Chan and Chester Jones, 1967; Oide and Utida, 1968b; Chester Jones, Chan and Rankin, 1969). The Corpuscles of Stannius also appear to respond to changes in the salinity of the medium. In Astyanax mexicanus, prolonged immersion in a one per cent sodium chloride solution results in signs of corpuscular atrophy (Rasquin, 1956). But in sea-water adapted European eels, Anguilla anguilla, histological examination reveals that the cells of the Corpuscles of Stannius have enlarged nuclei and other characteristic signs of increased activity (Olivereau, 1964; Hanke and Chester Jones, 1966; Hanke et al, 1967).

In salt-water fish the reverse is found on adaptation to fresh-water. Migrating adult Coho salmon show an increase in glomerular filtration rate and urine flow (Miles, 1971). In salt-water adapted eels transferred to fresh-water glomerular filtration rates increase as do urine flow rates (Ball et al, 1971). The marine form of the three-spined stickleback, Gasterosteus aculeatus L., shows an increase in glomerular size, an increase in glomerular

filtration rate and an increase in urine flow rates when transferred to fresh-water (Ogawa, 1968).

These changes may be explained in terms of water or electrolyte retention. In sea-water the fish secrete small amounts, with respect to fresh-water adapted fish, of a blood hypotonic urine in order to conserve water (Sharratt, Chester Jones and Bellamy, 1964a). This would require a reduced glomerular filtration rate or increased tubular water reabsorption. Since a linear correlations have been established between systemic blood pressure, urine flow rates and glomerular filtration rates (Rankin, Chan and Chester Jones, 1967; Butler, 1969), this could explain the function of the decrease in blood pressure. Drinking rates also increase in sea-water as does the permeability of the gut to water (Maetz and Skadhauge, 1968; Oide and Utida, 1968 a,b). In fresh-water the problem is electrolyte retention, therefore large amounts of dilute urine are excreted.

The Corpuscles of Stannius appear to have a diuretic effect on the kidney (Butler, 1969) but if this is an inhibitory effect is not known. However, it has been suggested that the secretions of the Corpuscles of Stannius promote renal tubular secretion of calcium (Rankin, Chan and Chester Jones, 1967).

In summary, fresh-water adapted fish have a higher blood pressure, greater glomerular filtration rates and greater urine flow rates than do the sea-water adapted eels. Adaptation to sea-water is accompanied by a decrease in these three parameters;

i.e. blood pressure, glomerular filtration rate and urine volume. The Corpuscles of Stannius are implicated in blood pressure since Stanniectomy results in a decrease in blood pressure in both fresh-water adapted and salt-water adapted eels (Chan et al, 1967). However, it appears that adaptation to sea-water results in corpuscular hypertrophy in the eel, Anguilla anguilla. This suggests that the corpuscular hypertrophy is not associated with increased release of a pressor substance in that blood pressure decreases on adaptation to sea-water. However, the common factor which is a result of Stanniectomy, apart from the decrease in blood pressure, of both fresh-water adapted and salt-water adapted eels is the hypercalcemic response. It is possible that it is this hypercalcemia which is causing the decrease in blood pressure and the other effects which follow Stanniectomy and that the effects result from the abnormal plasma and tissue fluid calcium levels.

5. CALCIUM AND MUSCLE CONTRACTION.

The presence of calcium ions has been shown to be essential for muscular contraction and relaxation (Ebashi, 1961; Ebashi and Lipmann, 1962; Hasselbach, 1964; Podolsky and Constantin, 1964; Sandow, 1965; Brady and Tan, 1966). Briefly, muscle contraction is believed to occur in the following manner: when the membrane of the muscle fiber is depolarized as a result of a stimulus the vesicles and tubular elements of the sarcoplasmic reticulum release previously bound calcium. The released free calcium then binds with

the actomyosin of the myofibrils causing a shrinkage of the actomyosin complex i.e. muscle contraction (Ebashi, 1961; Sandow, 1965; Lee et al, 1966). The calcium is recaptured by the sarcoplasmic reticulum when the membrane of the muscle fiber becomes repolarized (Ebashi, 1961; Sandow, 1965). This allows the actomyosin complex of the myofibrils to lengthen (Ebashi, 1961, Sandow, 1965) and results in relaxation of the muscle itself.

It has also been shown that the calcium for contraction and relaxation is stored in the sarcoplasmic reticulum (Ebashi and Lipmann, 1962). Skeletal muscle can store approximately ten times as much calcium as can cardiac or smooth muscle cells (Hasselbach, 1964). The decreased uptake (recapture by the sarcoplasmic reticulum) of calcium by the myofibrils when excitation is over appears to be due to a calcium "sink", i.e. a mechanism for reducing the amount of calcium available to the myofilaments (Podolsky and Constantin, 1964).

From this system it would appear that hypercalcemia would result in tetany and hypocalcemia would result in a loss of contractility. However, the reverse situation is found to be true. Experimental evidence indicates that the intracellular concentration of calcium in the muscle fiber itself determines contractility (Sandow, 1965). The control of intracellular calcium appears to be an active process which requires ATP (Ebashi and Lipmann, 1962; Fairhurst, Paulus and Jenen, 1967; Romero and Wittam, 1971). As well, a rise in intracellular calcium results in a decrease in

intracellular potassium, the rise probably being due to a loss of cellular ATP (Romero and Wittam, 1971). So, despite the fact that the fall in intracellular potassium results in a more easily depolarized cell membrane, there is no contraction of the muscle since ATP is also required for muscle contraction (Sandow, 1965).

The effect of calcium on the nervous system is somewhat different. It has been found that both sodium and calcium compete for sites in the membrane of the neuron but that these sites have a greater affinity for calcium than for sodium (Dubois and Bergman, 1971). Thus, hypercalcemia would result in an inhibition of saltatory conduction while hypocalcemia would result in membrane depolarization. Also, hypercalcemia tends to cause structural and functional changes of the neuromuscular junction (Heuser, Katz and Miledi, 1971) in such a way as to cause irreversible loss of response to membrane depolarization (op.cit., 1971). Thus hypercalcemia would not only result in a loss of contractility in muscle fibers but an inhibition of nervous conduction and would tend to inhibit passage of the impulse to the target muscle across the neuromuscular junction.

Cardiac muscle, like skeletal muscle, requires calcium for contraction (Brady and Tan, 1966; Friedman et al, 1967; Winegrad, 1971). Hypocalcemia results in a loss of contractility and an increase in electrical discharge (Marshall, 1961; Brady and Tan, 1966) while prolonged exposure to hypocalcemic conditions has been found to result in ventricular fibrillation (Marshall, 1961). Hypercalcemia also results in a slowing of the heart but in a different

fashion. Hypercalcemic conditions result in an increase of threshold potential at the pacemaker fibers of the sinoatrial node which results in a decreased heart rate (Marshall, 1961). Prolonged exposure to hypercalcemic conditions results in the heart stopping in a state of sustained contraction known as calcium rigor (op.cit., 1961).

Vascular smooth muscle also requires calcium for the process of contraction (Axelsson et al, 1967; Baudouin et al, 1972). However, hypercalcemia or hypocalcemia result in a loss of contractility in vascular smooth muscle (Sitrin and Bohr, 1971) and disturbances in the electrical activity of the vascular smooth muscle (Axelsson et al, 1967).

SUMMARY

Hypocalcemia or hypercalcemia would thus result in a loss of contractility or electrical activity in muscle fibers. Since both the cardiac muscle and the vascular smooth muscle are affected by high or low calcium levels this would result in a decreased cardiovascular efficiency and, as a result, a decrease in blood pressure.

Since the primary effect of Stanniectomy appears to be a massive increase in plasma and muscle calcium it is possible that the effect of Stanniectomy on blood pressure is not due to the loss of a pressor substance but is the result of a secondary effect brought about by altered plasma calcium levels. Accordingly, the effect of plasma calcium levels and Stanniectomy on blood pressure in the eel was investigated.

MATERIALS AND METHODS

1. EXPERIMENTAL ANIMALS

Fresh-water adapted yellow female eels procured from a commercial fish-dealer in Quebec city were used throughout this study. The average weight of these eels was approximately one kilogram. All eels were held for at least three weeks prior to use in tanks containing two hundred liters of dechlorinated Ottawa city tap-water to which seventy grams of sodium chloride crystals were added. Occasionally, 10 drops of a 1% aqueous solution of malachite green were added to inhibit fungal growth. The eels were not fed. The photoperiod and temperature regimens were not controlled. The water in the tanks was changed every two days to prevent accumulation of dissolved ammonia and to reduce the incidence of bacterial and fungal infections.

2. SURGICAL TECHNIQUES

A) ANAESTHETIZATION

Fish were anaesthetized by placing them in an aqueous solution (2gm/liter) of ethyl-m-aminobenzoate methane sulfonic acid tricaine (MS-222) until the eel was observed to have lost its righting response. The induction time for anaesthetization varied with the size of the fish but was routinely less than fifteen minutes.

B) TAGGING

Fish were tagged for all long term experiments and in those experiments which required identification of individual fish.

Tagging was accomplished by the method of Vladykov (1970). The tag consisted of a piece of numbered yellow plastic on a metal ring which was split diagonally (split-ring and plate type tag). The mouth of the eel was opened and the opened ring was pushed through the skin of the lower jaw so that the jawbone was encircled by the ring.

It was observed that the tagged fish had little difficulty in ventilating their gills and the tags remained firmly attached for the period of time required. One disadvantage of this method was that the wound caused by the ring did not always heal in the immediate vicinity of the ring. In several cases, marked necrosis of the surrounding tissue was noted at the site of the ring. Nevertheless, this method of tagging proved to be the most satisfactory of several techniques tested, at least so far as the present experimental requirements.

C) CANNULATION TECHNIQUES

The procedure used for cannulation was a modified version of the technique employed by Chester Jones et al, (1966) using the pneumogastric artery for blood pressure measurement and fluid withdrawal and the pneumogastric vein for fluid infusion or injection. The anaesthetized fish was placed ventral side up on the operating table and an incision was made in the mid-ventral abdominal wall extending from the posterior border of the heart, located by palpitation, to a point approximately two centimeters caudal to the posterior border of the liver. The exposed liver was reflected laterally to expose

the dorsal aorta, the cardinal veins, a pair of segmental arteries, the pneumogastric artery and vein, and the esophagus.

The pneumogastric artery was located and exposed by blunt dissection. The artery was tied off at a point immediately proximal to the point of bifurcation of the artery. An arterial clamp was placed on the vessel at its point of exit from the dorsal aorta. A suitable length (1 meter) of polyethylene tubing (Intramedic; PE-50) was attached at one end to a 26 gauge hypodermic needle. The needle, in turn, was installed on a 2 1/2 cc disposable syringe previously filled with a heparinized 0.6% saline solution. The PE tubing was then filled with the saline solution by depressing the syringe plunger until the solution dripped freely from the open end. This end was inserted into a small "V"-shaped incision in the pneumogastric artery and was tied firmly with No. 10 cotton thread. The arterial clamp was removed and the syringe plunger was pulled back until blood was observed in the cannula. The preparation was subsequently examined for air bubbles. If no bubbles were observed the cannula was tacked to the body wall by means of No. 10 cotton thread.

The pneumogastric vein was cannulated in a similar manner. The fascia surrounding the vein was removed by blunt dissection. The vein was ligated with No. 10 cotton thread at the posterior end of the cleared section. The anterior end of the vein was left open to allow free blood flow to prevent collapse of the vein. This arrangement allowed blood to be forced back manually to

reveal the incision made in the vein and to facilitate the insertion of the cannula. The cannula was tied securely into place and was tacked to the body wall by means of No. 10 cotton thread. The venous cannula was identical to the arterial cannula except that it was filled with non-heparinized 0.6% saline.

Both cannulas were lead out of the incision in the body wall. The incision was closed with a continuous suture using No. 10 cotton thread and the cannulas were secured to the dorsal fin by two sutures. Fish possessing both the pneumogastric artery and the pneumogastric vein cannulas will henceforth be referred to as double-cannulated eels. The deviations from the Chester Jones procedure were: heparin was not injected into the animal, the pneumogastric rather than the hepatic portal vein was cannulated, and the cannulas were anchored to the dorsal fin for extra strength.

Following cannulation, the fish were placed in plexiglass troughs measuring 83 cm long, 11.5 cm in width, 13 cm deep, and filled to a depth of 8 cm.

D) INFUSION TECHNIQUE

The infusion apparatus consisted of a ten milliliter disposable syringe driven by a Sage Infusion Pump, Model 352*. The infusion solutions consisted of 500 meq/l calcium chloride solution, 50 meq/l calcium gluconate solution, 0.6% sodium chloride solution, 6 gm/l tetra sodium EDTA solution or 10% sodium citrate

* Sage Instrument Company, A Subsidiary of Orion Research, Cambridge, Mass.

solution. In all cases, the solvent phase of the solution consisted of demineralized water. Animals were infused at the rate of 2.0 ml/kgm body weight/hour for all test solutions.

E) STANNIECTOMY

The technique used for the surgical removal of the Corpuscles of Stannius (Stannectomy or Stanex) was similar to that described by Butler (1969). But in the present study, to facilitate haemostasis, a piece of gelfoam (Upjohn Co.) was placed on the kidney covering the site where the corpuscles were removed. Further, the incision was closed with a continuous suture using No. 10 cotton thread rather than stainless steel wire. Sham-operations were performed in an identical manner but the Corpuscles of Stannius were not removed.

F) BLOOD COLLECTION

Blood was collected with a 2.5 ml disposable syringe fitted with a one inch, 26 gauge hypodermic needle. The heparinized saline was withdrawn from the arterial cannula into the syringe. The needle was removed from the cannula and four drops of blood allowed to flow out of the open end of the cannula. The needle in the collecting syringe was then inserted into the cannula and a one milliliter blood sample was drawn into the syringe. The blood was immediately transferred to a previously heparinized* ten milliliter glass stoppered centrifuge tube. The blood volume in the eel was

* Ammonium Heparin, A.H. Thomas, Philadelphia.

restored by injecting one milliliter of fresh-water teleost physiological solution via the arterial cannula. The cannula was subsequently refilled with heparinized 0.6% saline.

The blood was centrifuged at $766 \times g$ for 10 minutes; plasma was collected and stored frozen at -10°C in one milliliter disposable tuberculin syringes. The blood cells were discarded.

3. ANALYTICAL TECHNIQUES

A) BLOOD PRESSURE MEASUREMENT

Blood pressure was measured by connecting the arterial cannula to a Statham P23AC transducer*. Recordings were obtained on a Grass polygraph, model 5D**. The Grass polygraph was calibrated to a sensitivity of one millimeter pen deflection for each one millimeter of mercury pressure change.

B) TOTAL PLASMA CALCIUM AND SODIUM MEASUREMENTS

Total plasma calcium and total plasma sodium were measured by means of a Jarrel-Ash atomic absorption, flame emission, flame photometer (Atomsorb-Model 82-720)***. Total plasma calcium was measured using atomic absorption and plasma sodium was measured using flame emission. All standards were prepared in the lab but were checked

*Statham Laboratories Inc., Hato Rey, Puerto Rico.

**Grass Instrument Company, Quincy, Mass.

***Jarrel-Ash, Waltham, Mass.

against commercial plasma standards.

The plasma samples were brought to room temperature and a 0.1 ml aliquot of plasma was diluted with 1.0 ml of an aqueous solution of lanthium chloride (6,300 meq lanthium per liter). The lanthium served to suppress ionization interference by providing an excess of free electrons thereby preventing the ionization of the calcium. The aqueous lanthium chloride solution was also used as the blank preparation. The calcium concentration was read directly off the meter scale; sodium was read as per cent transmittance which was converted to optical density and read off a standard curve.

C) PLASMA IONIC CALCIUM

Plasma ionic calcium was measured using an Orion specific calcium ion electrode. The flow-through system, model 99-20, was chosen to eliminate exposure of the plasma to the atmosphere and the subsequent pH changes which could have occurred. This system consists of a syringe pump, model 88-01, a calcium flow-through electrode, and a flow-through reference electrode*. The millivolt potentials generated in the electrodes were read with an Orion, model 801, digital pH meter** or an Acumet pH meter model 340***. A major problem with this technique is that it is extremely difficult to prepare a standard with an ionic background identical to that of the plasma tested.

Nevertheless, the values recorded for ionic calcium concurred reasonably.

*Orion Research Inc., Cambridge, Mass.

**Orion Research Inc., Cambridge, Mass.

***Fisher Scientific

well with those reported previously in eels but measured by different technique (Butler, 1969).

4. EXPERIMENTAL PROCEDURES

A) EFFECT OF STANNIECTOMY AND ENVIRONMENTAL CALCIUM ON PLASMA CALCIUM LEVELS AND BLOOD PRESSURE.

These experiments were carried out by placing ten Stanniectomized, ten sham-Stanniectomized and ten unoperated control fish in dechlorinated Ottawa tap-water and an equivalent number of the same groups in an acalcemic 0.05% aqueous solution of sodium chloride.

After two weeks the animals were removed and the pneumogastric arterial cannula was installed as described previously. The eels were then transferred to the plexiglass troughs described previously and containing the same medium as the animals were held in post-operatively, where they were allowed to recover from the anaesthesia. Their blood pressure was recorded at five minute intervals for one hour. A 2.5 ml blood sample was then taken. The eels were killed by decapitation. Autopsies were performed to confirm the absence of the Corpuscles of Stannius. The blood plasma was analyzed for total and ionic plasma calcium levels and plasma sodium levels.

B) EFFECT OF CALCIUM CHLORIDE AND CALCIUM GLUCONATE INFUSION
ON PLASMA CALCIUM LEVELS AND ITS RELATION TO BLOOD PRESSURE

The first group was a control group and ten double-cannulated eels were used. An initial one milliliter blood sample was taken and the blood pressure recorded for two hours. A second one milliliter blood sample was taken and the blood pressure recorded for an additional two hours. A third and final one milliliter blood sample was taken and the animals were killed.

The blood plasma was analyzed for total and ionic plasma calcium levels and plasma sodium levels. Blood pressures were calculated from the recordings at the beginning and the end of each two-hour period of blood pressure recording.

Four other groups of ten fish were used and the procedure is summarized in Table III. In all four groups blood samples were taken before and after the period of calcium infusion. Blood pressures were calculated before and after the period of calcium infusion as well as before and after the period of saline infusion.

C) EFFECT OF ACUTE ARTIFICIALLY INDUCED HYPOCALCEMIA ON
BLOOD PRESSURE

The first group of this experiment consisted of ten double-cannulated eels which were placed in distilled water. Distilled water was used to prevent any uptake of calcium from the medium. This group served as a control group.

A one milliliter blood sample was taken following which blood pressure was recorded continuously for two hours. Subsequently,

TABLE III
SUMMARY OF PROCEDURE FOLLOWED IN HYPERCALCEMIA EXPERIMENT AND DATA OBTAINED

TYPE OF EEL USED	OPERATION PERFORMED	TEST SOLUTION INFUSED AND TIME OF INFUSION	DATA OBTAINED
Group two 24-Hour Stanex fish	Double cannulation	Calcium Gluconate - 2 hours 0.6% Saline - 2 hours	Total plasma Calcium levels Blood pressures
Group three Intact fish	Double cannulation	Calcium Gluconate - 2 hours 0.6% Saline - 2 hours	Total plasma Calcium levels Blood pressures
Group four Intact fish	Double cannulation	Calcium Chloride - 2 hours 0.6% Saline - 2 hours	Total plasma Calcium levels Blood pressure
Group five Intact fish	Double cannulation	Calcium Chloride - 2 hours 0.6% Saline - 2 hours	Total plasma Calcium levels Blood pressures

a second one milliliter blood sample was taken and blood pressure was again recorded continuously for two hours. A third and final one milliliter blood sample was taken and the eels were then killed by decapitation.

The blood plasma was analyzed for plasma sodium levels, total plasma calcium and plasma ionic calcium levels. Blood pressures were calculated before and after each of the recording periods.

The procedures used for the other two groups in this experiment are summarized in Table IV. Blood samples were taken before and after the period of EDTA infusion or citrate infusion. Blood pressures were calculated before and after each infusion period.

Theoretically, one mole of EDTA complexes one mole of ionic calcium (Fischer and Peters, 1968). However, this is only obtained at a pH of 10.0. At lower pH, the efficiency of EDTA as a chelating agent tends to decrease (Fischer and Peters, 1968). It was calculated that 4.0 ml of a 6 gm/liter solution of EDTA would complex approximately 66% of the plasma ionic calcium of a one kilogram eel. The figure of 66% was a purely arbitrary choice and was made on the assumption that if 66% of the plasma ionic calcium was chelated, an effect on blood pressure would be observed but the animal would not die from lack of ionic calcium. It was observed that blood samples drawn after EDTA infusion were easily haemolyzed; this did not occur after citrate infusion. It was also observed that citrate appeared to complex calcium ions more efficiently than

TABLE IV

SUMMARY OF PROCEDURE FOLLOWED IN HYPOCALCEMIA EXPERIMENT AND DATA OBTAINED

TYPE OF EEL USED	OPERATION PERFORMED	TEST SOLUTION INFUSED AND TIME OF INFUSION	DATA OBTAINED
Intact fish	Double cannulation	EDTA - 2 hours 0.6% Saline - 2 hours	Plasma Ionic Calcium levels Blood pressures
Intact fish	Double cannulation	Sodium citrate - 2 hours 0.6% Saline - 2 hours	Plasma Ionic Calcium levels Blood pressures

EDTA at blood pH and so citrate was used in place of EDTA despite the fact that a more concentrated solution (citrate - 10%, EDTA 0.6%) was required.

D) EFFECT OF HYPOCALCEMIA FOLLOWED BY HYPERCALCEMIA ON BLOOD PRESSURE

Ten double-cannulated eels were used in this experiment. Hypocalcemia and hypercalcemia were induced by infusing 10% sodium citrate and 500 meq/l calcium chloride solutions respectively.

A one milliliter blood sample was taken and the fish were infused with sodium citrate for a two-hour period. Blood pressures were recorded continuously. After the period of citrate infusion, a second one milliliter blood sample was taken. The fish were then infused with calcium chloride solution with continuous monitoring of blood pressure. A terminal one milliliter blood sample was taken following this infusion period and the fish were killed by decapitation.

The plasma samples were analyzed for total plasma sodium levels, total plasma calcium and plasma ionic calcium levels. Blood pressures were calculated before and after each infusion period.

E) EFFECT OF ANGIOTENSIN II AND CORPUSCULAR EXTRACT ON PLASMA CALCIUM LEVELS AND BLOOD PRESSURE

Three solutions were used in this group, an angiotensin II solution, a saline extract of the Corpuscles of Stannius and an isotonic (0.6%) saline solution.

One milligram of synthetic angiotensin II* was dissolved in one liter of isotonic saline. Ten milliliters of this solution were taken and diluted ten times with isotonic saline to give a final concentration of $0.1 \mu\text{g/ml}$. This was the concentration used for injection purposes.

The saline extract of the Corpuscles of Stannius was made by homogenizing 20 mg of corpuscular tissue in an equal number of milliliters of cold isotonic saline using a glass, hand-driven homogenizer. The homogenate was filtered through Whatman No. 1 filter paper. The filtrate was used without further preparation.

The isotonic saline was prepared by dissolving six grams of sodium chloride crystals in one liter of demineralized water.

Eight double-cannulated eels were used in this experiment. The fish were allowed to recover from anaesthesia in dechlorinated tap-water and an initial one milliliter blood sample was taken. The fish were injected through the venous cannula, with $0.1 \mu\text{g/kg}$ body weight of the angiotensin II solution and blood pressure was recorded continuously for the following hour. A second one milliliter blood sample was taken. The fish were then injected with 1.0 ml/kg body weight of the saline extract of the Corpuscles of Stannius. Blood pressure was recorded continuously for one hour after which a third one milliliter blood sample was taken. An equivalent amount of isotonic saline was then injected as a control and blood pressure was recorded for a further one hour.

*Sigma Chemical Co.

Plasma samples were analyzed for total plasma sodium, total plasma calcium and plasma ionic calcium levels. Blood pressures were calculated before and one hour after the injections.

5. STATISTICAL PROCEDURES

For the purpose of this study, a probability of P 0.05 was taken as significant and a probability of P 0.01 was taken as highly significant. For sample comparison involving three or fewer means, the Students "t" test was performed. For comparisons involving more than three means, one way or two way analysis of variance were carried out on the data. Significance of difference between pairs of means were then tested with multiple means comparison tests (Tukey's W). Linear regression analysis were performed to determine least squares lines. Nonparametric Spearman's rank correlation coefficient, r_s , was used to judge the significance of correlation between values. This latter technique was used as valid assumptions concerning the shape of the populations from which the data were drawn were not possible.

RESULTS

1. EFFECT OF STANNIECTOMY AND ENVIRONMENTAL CALCIUM ON PLASMA
CALCIUM LEVELS AND BLOOD PRESSURE

A) EELS ADAPTED TO TAP-WATER

Data concerning the effects of Stanniectomy on plasma total and ionic calcium levels and on blood pressure in tap-water adapted eels are summarized in Table V. These data clearly indicate that the surgical removal of the Corpuscles of Stannius is followed by significant alterations in total plasma calcium levels ($P < 0.01$) and blood pressure ($P < 0.05$). Compared to normal and sham-operated eels, the Stanniectomized eels were found to be hypercalcemic and hypotensive. Table V also shows that at least 50% of the increase in plasma calcium was contributed by the ionic calcium fraction. The sham-operation did not result in any significant changes in any of the parameters measured.

B) EELS ADAPTED TO ACALCEMIC WATER

The hypercalcemia and hypotension observed in tap-water adapted Stanniectomized eels (Table V) was not observed in eels adapted to an acalcemic environment (Table VI). The small, but significant increase in the total plasma calcium levels in the sham-operated eels (5.58 ± 0.23 meq/l) when compared to the intact controls (3.90 ± 0.24 meq/l) is considerably less than the near doubling of total plasma calcium seen in Stanniectomized fish adapted to tap-water. Significant changes in plasma ionic calcium in the

TABLE V
EFFECT OF STANNICTOMY ON PLASMA TOTAL AND IONIC CALCIUM LEVELS AND ON BLOOD
PRESSURE IN TAP WATER ADAPTED EELS ^a

GROUP	TOTAL PLASMA CA ⁺⁺ mEq/l	PLASMA IONIC CALCIUM mEq/l	BLOOD PRESSURE mm Hg
Stanniectomized	10.22 ± 0.95 (9) ^b	>4.0* ^b	23.5 ± 2.17 (8) ^b
Sham-operated	5.51 ± 0.39 (8)	1.36 ± 0.06 (8)	28.35 ± 1.14 (8)
Controls	5.28 ± 0.18 (8)	1.49 ± 0.06 (8)	30.34 ± 1.25 (8)

a all values are means ± S.E.M (N) ^b
 b P < 0.05 Stanex compared to Shams and Controls
 * Denotes minimum estimate of plasma ionic calcium levels

TABLE VI

EFFECT OF STANNICTOMY ON PLASMA TOTAL AND IONIC CALCIUM LEVELS AND ON BLOOD PRESSURE IN ACALCEMIC WATER ADAPTED EELS^a

GROUP	TOTAL PLASMA CA ⁺⁺ mEq/l	PLASMA IONIC CA ⁺⁺ mEq/l	BLOOD PRESSURE mm Hg
Stanniectomized	5.15 ± 0.43 (10)	1.79 ± 0.21 (10)	25.02 ± 1.33 (10)
Sham-Operated	5.58 ± 0.23 (8) ^b	1.66 ± 0.12 (8)	24.21 ± 1.41 (8)
Intact Controls	3.90 ± 0.24 (6)	1.46 ± 0.14 (6)	24.83 ± 0.89 (6)

a all values are means ± S.E.M. (N)

b P < 0.05 Shams compared to Intact Controls

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Stanniectomized and sham-Stanniectomized eels compared to the intact controls were not observed.

C) EFFECT OF TWENTY-FOUR HOUR STANNIECTOMY ON BLOOD PRESSURE AND TOTAL PLASMA CALCIUM LEVELS IN EELS ADAPTED TO TAP-WATER

Table VII shows the effect of twenty-four hour Stanniectomy on plasma calcium levels and blood pressure. No significant changes in plasma calcium levels or blood pressure were observed.

D) RELATIONSHIP BETWEEN PLASMA TOTAL OR IONIC CALCIUM ON BLOOD PRESSURE IN EELS ADAPTED TO EITHER TAP-WATER OR ACALCEMIC WATER

Figures 1a and 1b illustrate the relationship between total plasma calcium levels and blood pressure and between plasma ionic calcium levels and blood pressure respectively. The values are those previously used to calculate the means shown in Tables V and VI. In both cases, significant negative correlations ($P < 0.05$) between total plasma calcium and blood pressure (Figure 1a) and between plasma ionic calcium and blood pressure (Figure 1b) are found. Whilst it is recognized that a significant correlation does not denote a cause-effect relationship, the negative slope and significant correlation coefficients does suggest at least a relationship between plasma calcium levels and blood pressure.

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TABLE VII
TOTAL PLASMA CALCIUM AND BLOOD PRESSURE IN TAP WATER
ADAPTED EELS 24-HOURS AFTER STANNIECTOMY

GROUP	TOTAL PLASMA CALCIUM LEVELS mEq/l.	BLOOD PRESSURE mm Hg
Intact Controls	4.73 ± 0.11 (9)	27.0 ± 1.28 (9)
Stanniectomized	4.68 ± 0.17 (9)	24.67 ± 1.43 (9)

a values are means ± S.E.M. (N)

FIGURE 1a

Correlation between total plasma calcium levels and blood pressure in tap-water adapted and acalcemic water adapted American eels.

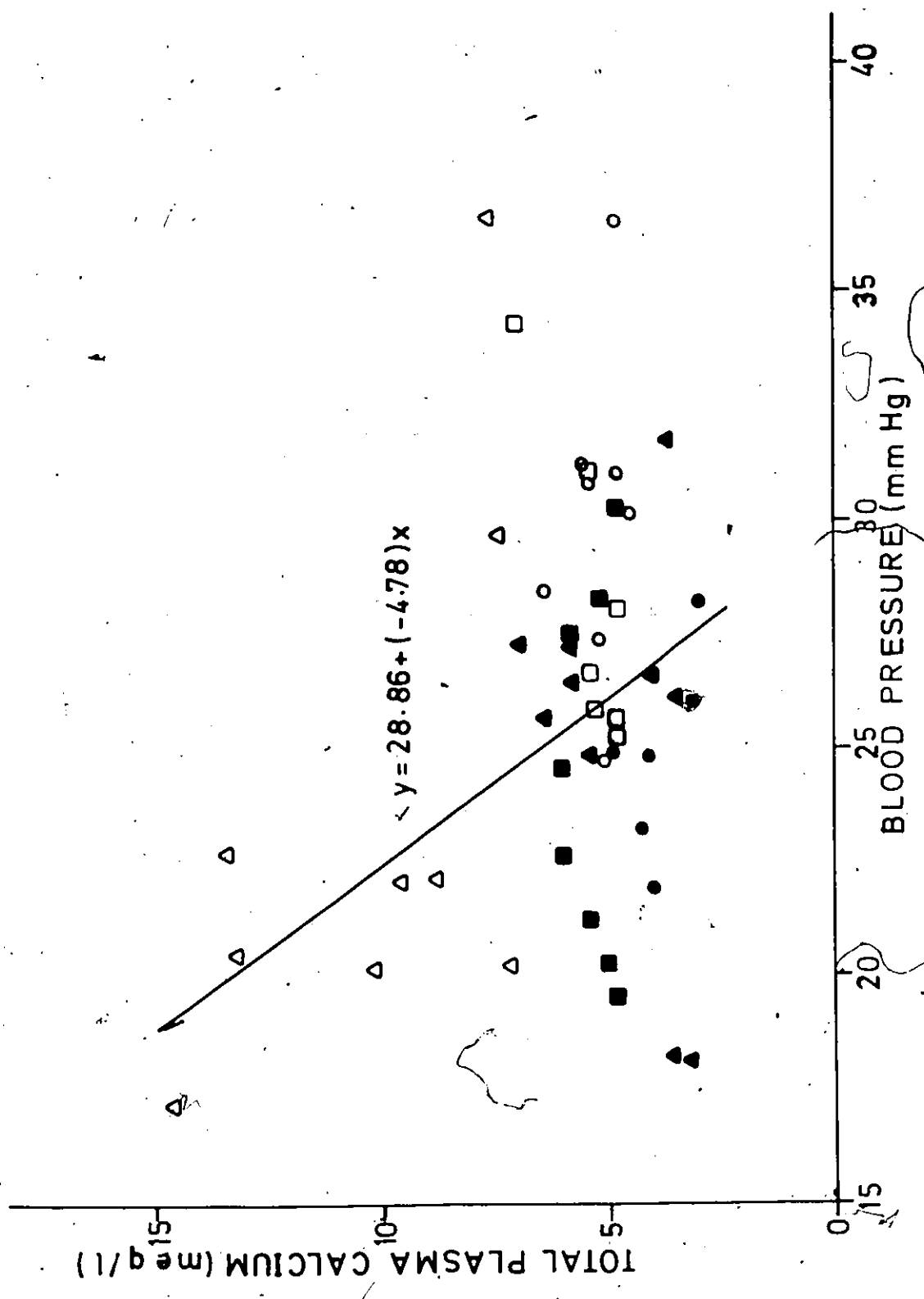
LEGEND

- △ - Tap-water adapted Stanniectomized eel
- ◻ - Tap-water adapted Sham-operated eels
- - Tap-water adapted intact control eels

- ▲ - Acalcemic water adapted Stanniectomized eels
- - Acalcemic water adapted Sham-operated eels
- - Acalcemic water adapted Intact control eels

For the line equation $y = 28.86 + (-4.78) x$, the correlation coefficient, $r = -0.6227$ ($P < 0.01$). The rank correlation coefficient (Spearman's r) $r = -0.4401$ ($P < 0.01$).





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FIGURE 1b

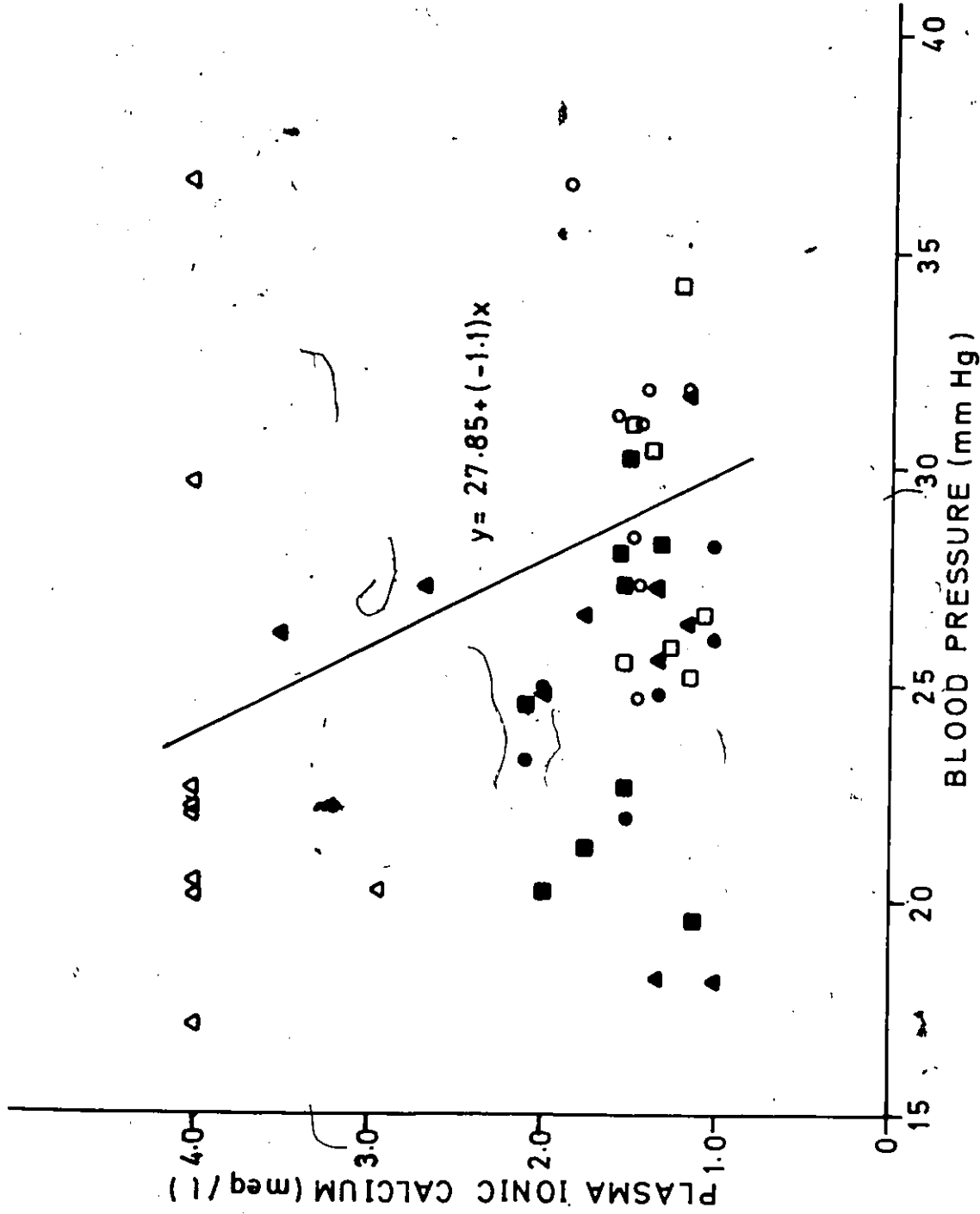
Correlation between plasma ionic calcium levels and blood pressure in tap-water adapted and acalcemic water adapted American eels.

LEGEND

- △ - Tap-water adapted Stanniectomized eels
- - Tap-water adapted Sham-operated eels
- - Tap-water adapted Intact control eels

- ▲ - Acalcemic water adapted Stanniectomized eels
- - Acalcemic water adapted Sham-operated eels
- - Acalcemic water adapted Intact control eels

For the line equation $y = 3.85 + (-1.1)x$, the correlation coefficient, $r = -0.4980$ ($P < 0.01$) and the rank correlation coefficient (Spearman's r) $r = -0.6645$ ($P < 0.01$).



2. EFFECT OF INDUCED HYPERCALCEMIA ON BLOOD PRESSURE IN EELS

A) CONTROL GROUP

The effects of the double-cannulation procedure on blood pressure and plasma calcium levels are summarized in Table VIII and Figure 2. The surgical sequelae appear to be non-significant, minimal increases in total and ionic plasma calcium levels, together with a slight decrease in blood pressure. The initial two-hour recording period (Period A-Table VIII) was characterized by a small decrease in blood pressure and small increases in total plasma calcium levels and plasma ionic calcium. These changes were not significant ($P > 0.10$).

During the second two hours (Period B-Table VIII) the blood pressure (Figure 2) and plasma calcium values remained unchanged. The small drop in blood pressure between Period A at T+120 min. and Period B at T+0 min. represents the effect of withdrawing one ml of blood. This drop was not significant ($P > 0.10$).

B) EFFECT OF CALCIUM INFUSION ON PLASMA CALCIUM LEVELS AND BLOOD PRESSURE

a) Calcium Gluconate - Infusion of calcium gluconate (50 meq/l) in intact fish resulted in a 32% increase in total plasma calcium ($P < 0.05$) but did not significantly affect the blood pressure ($P > 0.10$) (Table IX). Further, in this group of eels, no significant ($P > 0.10$) correlation could be established between total plasma calcium levels and blood pressure apart from twenty-four hour Stanniectomized

TABLE VIII

EFFECT OF THE DOUBLE CANNULATION PROCEDURE ON PLASMA CALCIUM LEVELS (mEq/l)

AND ON BLOOD PRESSURE (mm Hg) IN TAP WATER ADAPTED EELS ^a

PERIOD	TOTAL PLASMA CALCIUM AT T + 0 min. mEq/l	TOTAL PLASMA CALCIUM AT T + 120 min. mEq/l	PLASMA IONIC CALCIUM AT T + 0 min. mEq/l	PLASMA IONIC CALCIUM AT T + 120 min. mEq/l	BLOOD PRESSURE AT T + 0 min. mm Hg	BLOOD PRESSURE AT T + 120 min. mm Hg
PERIOD A	4.73 ± 0.11 (9)	4.71 ± 0.34 (9)	1.64 ± 0.06 (9)	1.72 ± 0.19 (9)	27.0 ± 1.28 (9)	25.22 ± 1.50 (9)
PERIOD B	4.71 ± 0.12 (9)	5.04 ± 0.49 (9)	1.72 ± 0.19 (9)	1.77 ± 0.11 (9)	24.89 ± 1.79 (9)	25.0 ± 1.69 (9)

a, values are means ± S.E.M. (N)

b Period A corresponds to the first two hours and Period B corresponds to the second two hours

FIGURE 2

Effect of the double-cannulation procedure on blood pressure during the experimental period.

The values are means \pm S.E.M.

The break in the line at T = 120 min. represents the end of the first two hour recording period (Period A) and the point where a one milliliter blood sample was taken. The second two hour recording period (Period B) begins at T + 120 min. on the graph for sake of continuity although there was a ten minute period required for taking the blood sample.

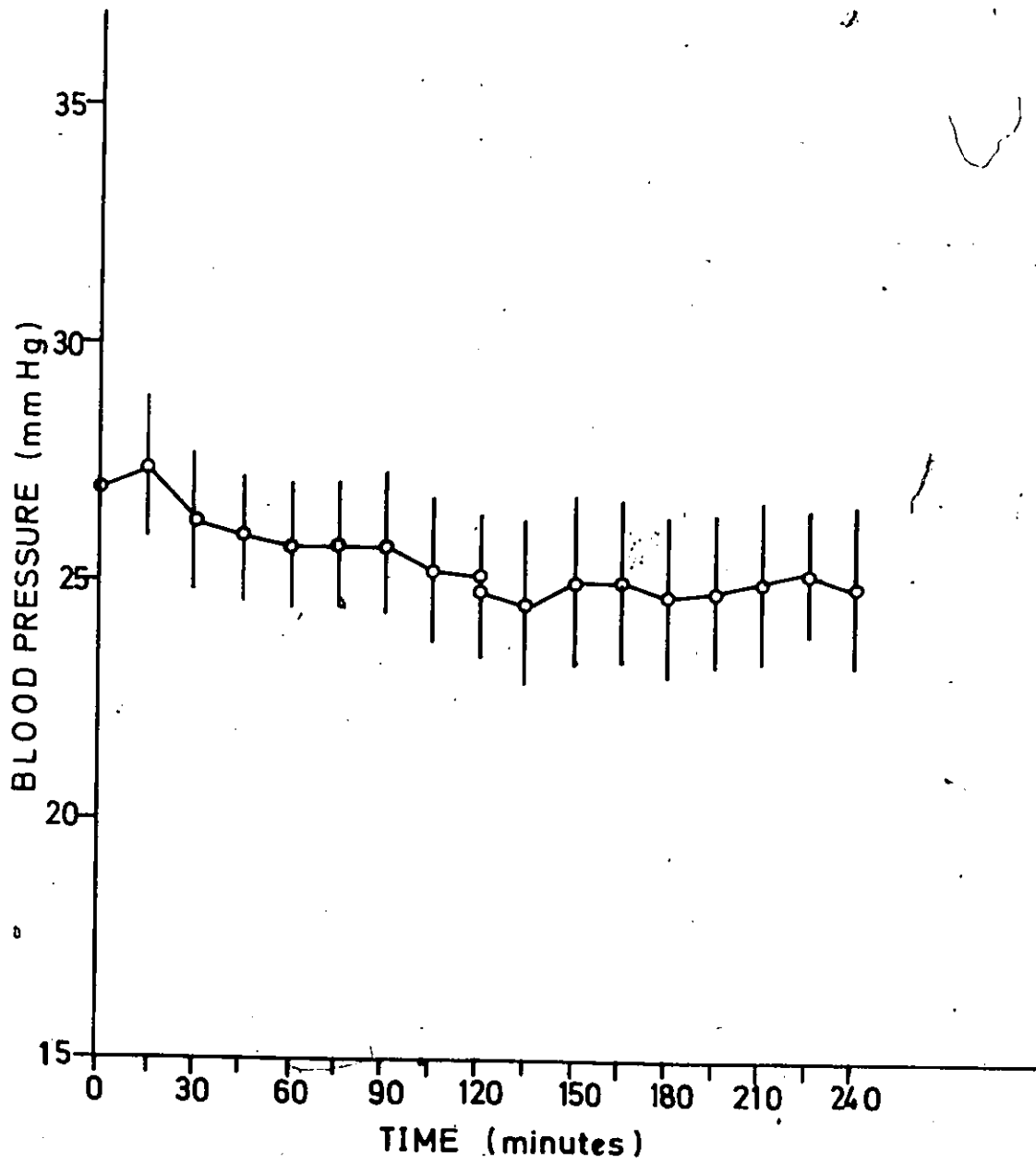


TABLE IX
 EFFECT OF CALCIUM GLUCONATE AND SODIUM CHLORIDE INFUSION ON TOTAL PLASMA
 CALCIUM AND BLOOD PRESSURE IN INTACT EELS ^a

TIME PERIOD	CALCIUM Total plasma calcium with calcium gluconate mEq/l	GLUCONATE INFUSION Blood pressure mm Hg	SALINE INFUSION Blood pressure mm Hg
Initial reading	5.60 ± 0.14 (9) ^b	29.72 ± 1.58 (9)	28.17 ± 2.56 (9)
After two hours of Infusion	7.40 ± 0.1 (9) ^b	30.28 ± 1.57 (9)	26.33 ± 2.57 (9)

^a values are means ± S.E.M. (N)

^b P < 0.05 (values obtained after infusion compared to those obtained before infusion)

eels (Figure 3).

Figure 4 shows the more detailed pattern of blood pressure response observed over the two-hour calcium infusion period. Blood pressure showed an initial rise which peaked at T+30 min. followed by a decline which reached the lowest point at T+60 min. A second peak was noted at T+90 min. and a second decrease at T+105 min. These individual variations were not, however, significant ($P > 0.10$).

b) Calcium Chloride - In the previous experiment, plasma calcium levels were increased but the increase was not of the same order of magnitude as that obtained following Stanniectomy (Table V). This suggested the fish were clearing the calcium as rapidly as calcium was infused so that a higher calcium infusion rate was deemed necessary. But due to the limited solubility of calcium gluconate at the required concentration (500 meq/l calcium), calcium chloride was used as the calcium donor.

The effect of infusing more calcium in the form of a concentrated calcium chloride solution was a massive increase ($P < 0.01$) in plasma calcium (Table X) to levels characteristic of two-week Stanniectomized eels (Table V). In addition the marked increase in plasma calcium was accompanied by a significant ($P < 0.05$) reduction in blood pressure to levels previously observed only in hypercalcemic two-week Stanniectomized eels (Table X and Table V).

A second calcium chloride infusion study confirmed these data (Table XI). Again a massive and highly significant ($P < 0.01$)

FIGURE 3

The effect of two-hour infusion of calcium gluconate on blood pressure and plasma calcium levels in intact eels and eels 24-hours after Stanniectomy.

LEGEND

▲ - Intact eels before infusion

● - Intact eels after infusion

X - 24-hour Stanniectomized eels before infusion

○ - 24-hour Stanniectomized eels after infusion

For the line equation $y = 29.73 + 0.04 x$, $r = 0.009$ ($P > 0.10$) and for the line equation $y = 32.35 + (-154) x$, $r = -0.4089$ ($P < 0.05$). In each case r is the correlation coefficient.

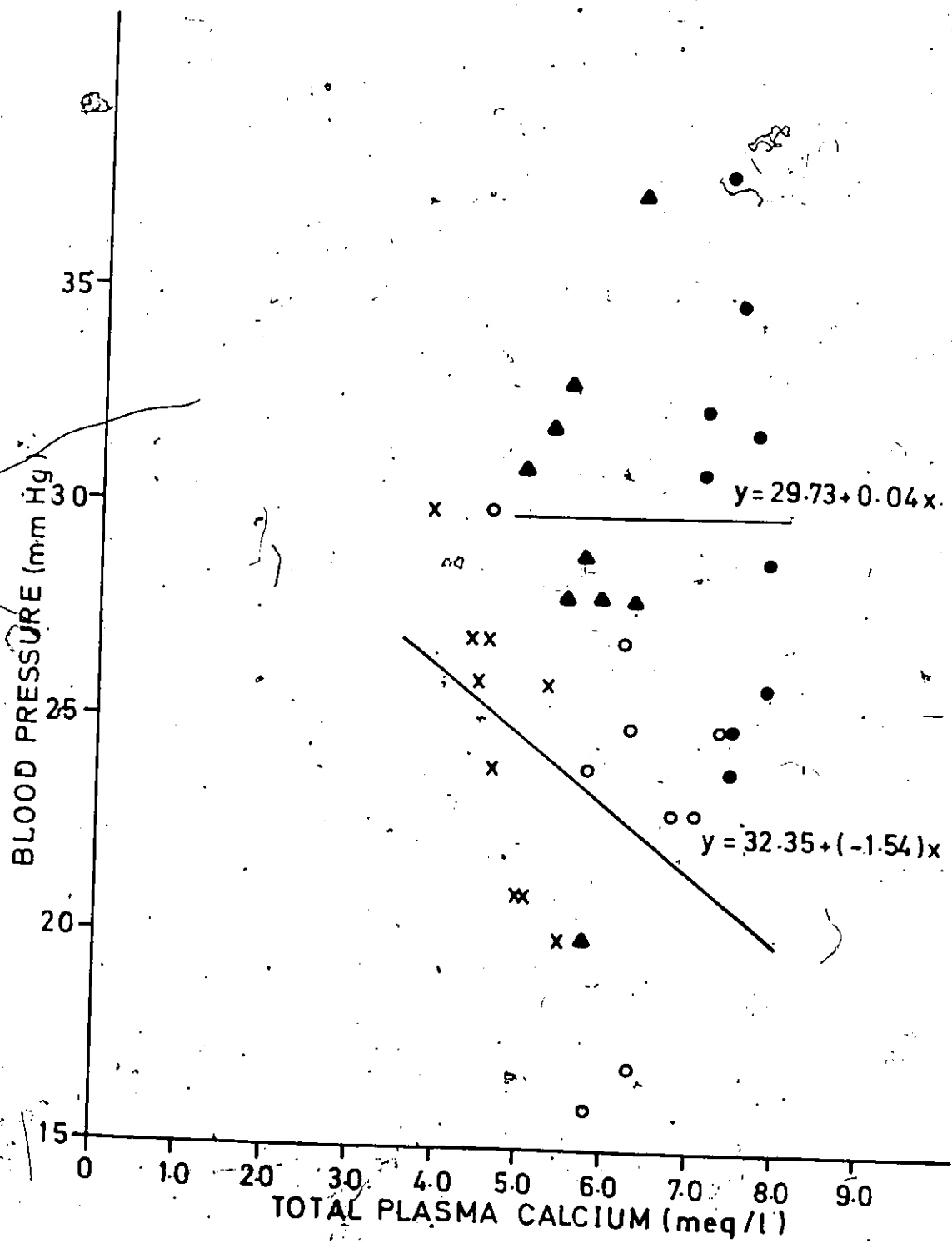


FIGURE 4

The effect of infusing calcium gluconate solution on blood pressure in intact eels adapted to tap-water.

Values are means \pm S.E.M.

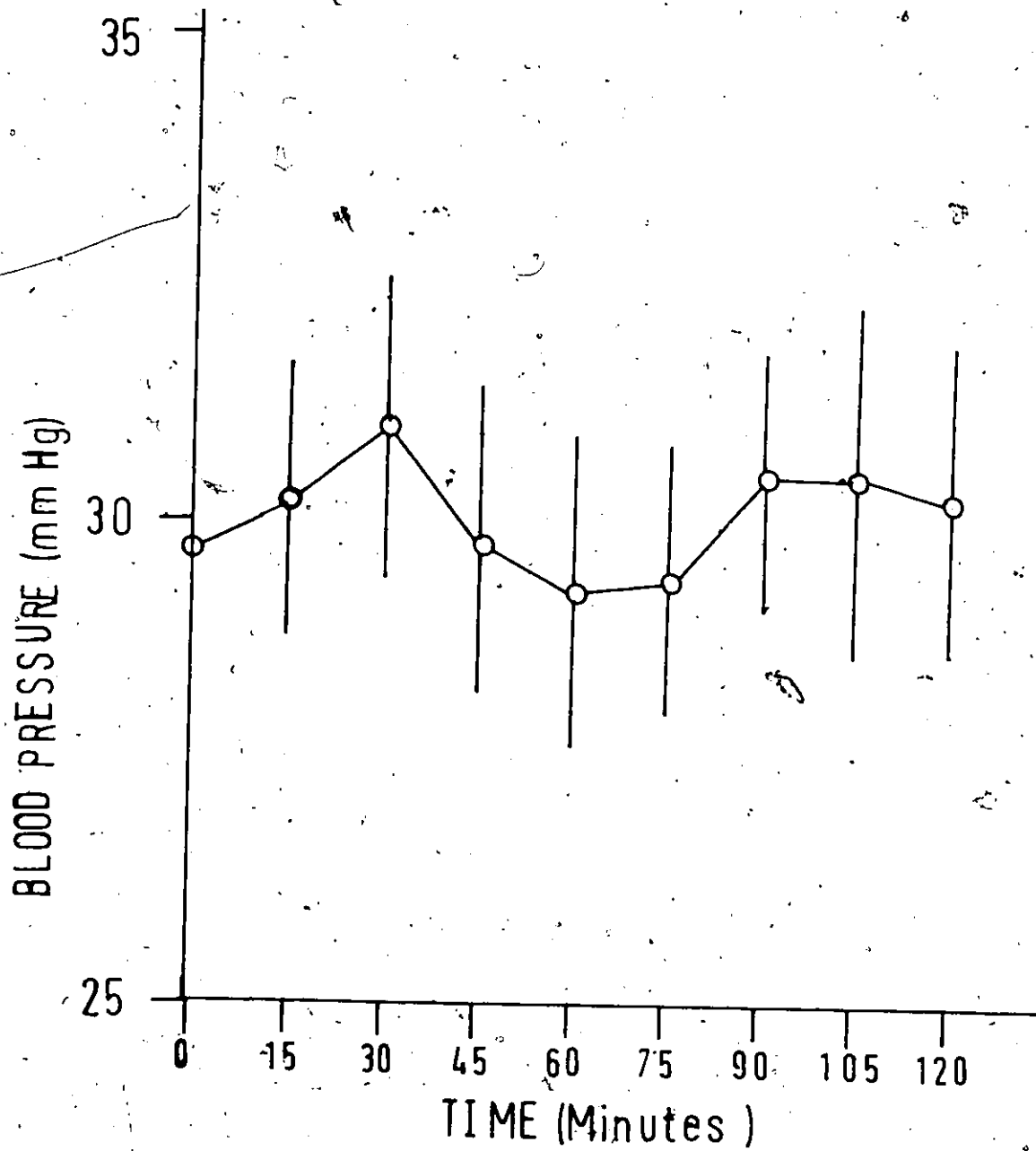


TABLE X
 EFFECT OF CALCIUM CHLORIDE INFUSION ON TOTAL PLASMA CALCIUM LEVELS AND
 BLOOD PRESSURE IN INTACT EELS ^a

PERIOD	TOTAL PLASMA CALCIUM mEq/l	CALCIUM INFUSION	BLOOD PRESSURE (mm Hg)
		CALCIUM INFUSION	SALINE INFUSION
Initial reading	5.89 ± 0.12 (10)	27.5 ± 1.50 (10)	24.8 ± 1.64 (8)
After two hours of Infusion _p	11.1 ± 0.84 (8) ^c	23.0 ± 2.00 (8) ^b	28.8 ± 2.2 (7) ^b

a values are means ± S.E.M. (N)

b P < 0.05

c P < 0.01

TABLE XI

EFFECT OF CALCIUM CHLORIDE INFUSION ON TOTAL PLASMA CALCIUM LEVELS AND BLOOD PRESSURE IN INTACT EELS ^a

PERIOD	TOTAL PLASMA CALCIUM mEq/l	CALCIUM INFUSION	BLOOD PRESSURE (mm Hg)	SALINE INFUSION
Initial reading	6.01 ± 0.15 (8)	26.3 ± 2.24 (8)	22.0 ± 3.2 (8)	
After two hours of Infusion	15.85 ± 1.36 (8)	21.1 ± 2.28 (8)	21.75 ± 3.5 (8)	

a. values are means ± S.E.M. (N)

b. P < 0.05

c. P < 0.01

increase in plasma calcium was observed. This increase in plasma calcium was accompanied by a large decrease in pneumogastric arterial blood pressure; the decrease in blood pressure was statistically significant ($P < 0.05$). The increase in plasma calcium and the decrease in blood pressure were even greater than those obtained in the previous calcium chloride study.

The results of these two experiments are combined in Table XII. The overall increase in total plasma calcium was found to be highly significant ($P < 0.01$) and a significant ($P < 0.05$) decrease in blood pressure accompanied the increase in total plasma calcium (Tables XIII and XIV). A significant ($P < 0.05$) negative correlation between plasma calcium and blood pressure was observed (Figure 5).

The more detailed response of blood pressure to the two-hour calcium chloride infusion studies is shown on Figure 6. In both the first and second infusion studies, blood pressure showed an initial rise which peaked at T+30 min. This initial increase was not significant ($P > 0.10$). Blood pressure then declined steadily until T+120 min. which was the end of the recording period, with the exception of the period from T+45 min. to T+60 min. during the first infusion study where blood pressure remained stable.

c) Sodium Chloride - In order to account for possible volume changes and other non-specific effects of the infusion, each of the preceding experiments incorporated a saline infusion control group. These data are found in Table XV, Table X, Table XI, Table XII and Figure 7. These data show that the overall effect of the

TABLE XII

EFFECT OF CALCIUM CHLORIDE INFUSION ON TOTAL PLASMA CALCIUM LEVELS AND

BLOOD PRESSURE IN INTACT EELS (Summed Data) ^a

PERIOD	TOTAL PLASMA CALCIUM mEq/l	CALCIUM INFUSION	BLOOD PRESSURE (mm Hg)
At T + 0 min. during infusion	5.99 ± 0.10 (18)	26.83 ± 1.29 (18)	23.44 ± 2.04 (16)
At T + 120 min. during infusion	13.48 ± 0.93 (16)	21.69 ± 1.91 (16)	24.67 ± 2.21 (15)

^a values are means ± S. E. M. (N)

^b P < 0.01

^c P < 0.05

TABLE XIII

TWO-WAY ANALYSIS OF VARIANCE OF BLOOD PRESSURE DATA FOR CALCIUM CHLORIDE
INFUSION EXPERIMENTS

SOURCE VARIATION	SUM OF THE SQUARES	MEAN SQUARE	DEGREES OF FREEDOM	F. RATIO
Treatment	150.16	75.08	2	13.98
Block	145.33	145.33	1	27.06
Interaction (Treatment x Block)	33.07	16.54	2	3.08
Error	230.87	5.37	43	---
TOTAL	559.43	---	48	---

TABLE XIV

TWO-WAY ANALYSIS OF VARIANCE FOR TOTAL PLASMA CALCIUM LEVEL DATA FOR
CALCIUM CHLORIDE INFUSION EXPERIMENTS

SOURCE VARIATION	SUM OF THE SQUARES	MEAN SQUARE	DEGREES OF FREEDOM	F RATIO
Treatment	516.05	516.05	1	146.19
Block	65.08	65.08	1	18.44
Interaction (Treatment x block)	24.77	24.77	1	7.02
Error	119.99	3.53	33	--
TOTAL	725.89	--	36	--

FIGURE 5

The effect of two-hour infusion of calcium chloride on blood pressure and total plasma calcium levels in intact tap-water adapted American eels.

LEGEND X - Intact eels before infusion

○ - Intact eels after infusion

For the line equation $y = 32.18 + (-0.77) x$, the correlation coefficient, $r = -0.5188$ ($P < 0.05$).

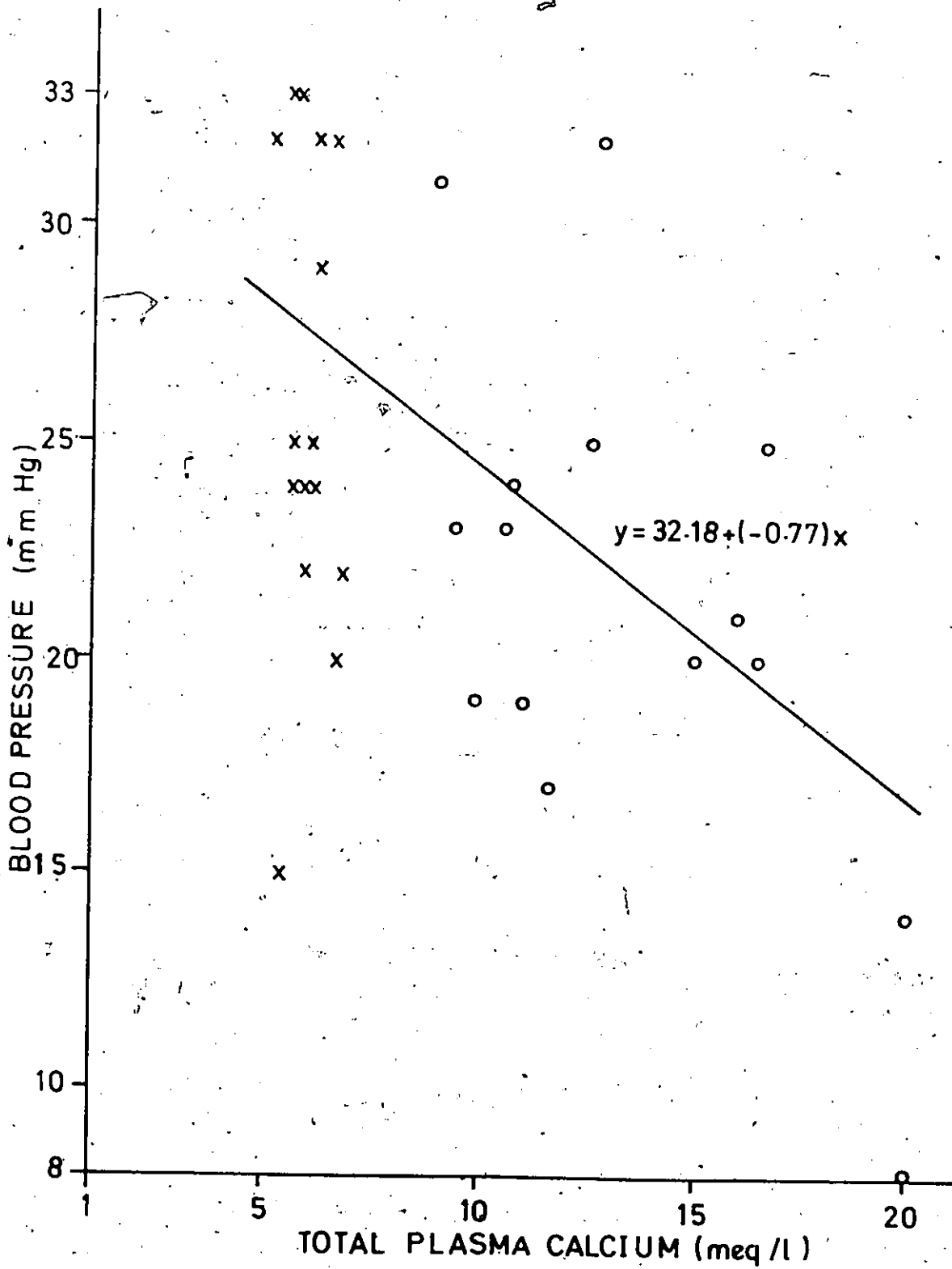


FIGURE 6

The effect of calcium chloride infusion on blood pressure in intact, tap-water adapted eels.

Values are means \pm S. E. M.

The unbroken line represent the first experiment and the broken line represents the second (confirmatory) experiment.

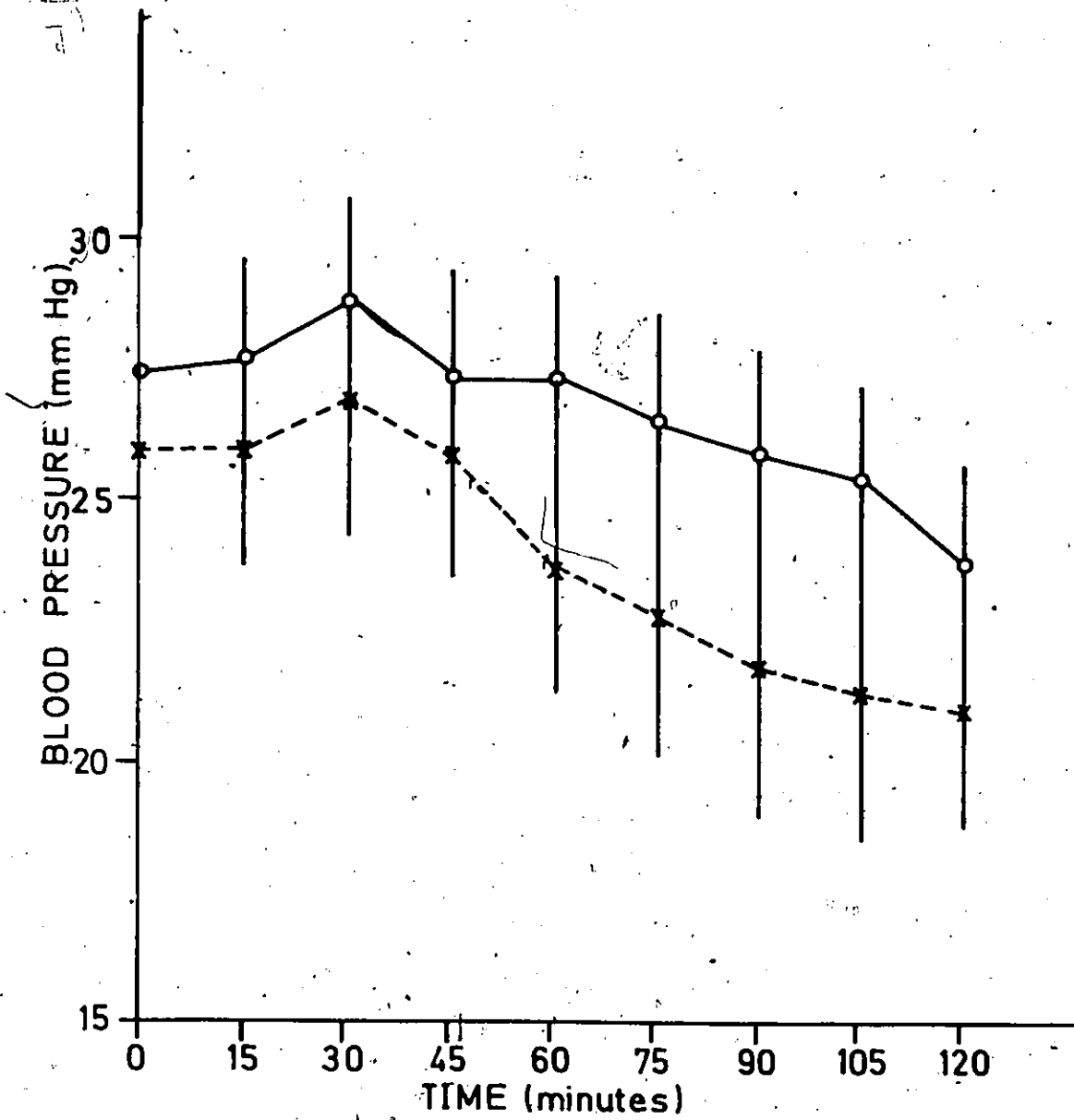


TABLE XV
 EFFECT OF CALCIUM GLUCONATE INFUSION ON TOTAL PLASMA CALCIUM AND BLOOD
 PRESSURE IN EELS 24 HOURS AFTER STANNICTOMY ^a

PERIOD	TOTAL PLASMA CALCIUM LEVELS mEq/l	CALCIUM INFUSION	BLOOD PRESSURE(mm Hg)	SALINE INFUSION
At T + 0 min	4.68 ± 0.17 (9)	24.67 ± 1.43 (9)	23.67 ± 1.85 (9)	
At T + 120 min	6.20 ± 0.29 (9) ^b	23.33 ± 1.48 (9)	22.89 ± 1.93 (9)	

^a values are means ± S.E.M. (N)

^b P < 0.05 comparisons are made in the vertical columns

infusion of a volume of sodium chloride equal to the volume of calcium chloride or calcium gluconate was minimal. The only significant effect of sodium chloride on blood pressure occurred during the first calcium chloride experiment when the NaCl infusion resulted in a significant ($P < 0.05$) increase in blood pressure. This tends to further support the hypotensive action of hypercalcemia as a similar volume of calcium chloride resulted in a significant decrease in blood pressure (Figure 7).

C) EFFECT OF CALCIUM GLUCONATE INFUSION ON TOTAL PLASMA

CALCIUM AND BLOOD PRESSURE IN TWENTY-FOUR HOUR

STANNIECTOMIZED EELS

Since it was suggested that the Corpuscles of Stannius have a calciuretic effect, an attempt was made to induce hypercalcemia by infusing calcium gluconate into eels deprived of their Corpuscles of Stannius. The results from this experiment are summarized in Table IV and Figures 3 and 8. As in intact fish, calcium gluconate infusion resulted in a 32% increase in total plasma calcium but the blood pressure was not significantly affected ($P > 0.10$). However, the data in Figures 3 and 8 do suggest that the overall effect of calcium gluconate infusion is a decrease in blood pressure with rising total plasma calcium. These data, however, are inconclusive. Again, saline infusion had no marked effect on blood pressure.

FIGURE 7

The per cent deviation from initial blood pressure values as a result of calcium chloride and isotonic saline infusion in intact eels. The solid line represents the calcium chloride infusion (x—x = calcium chloride) and the broken line represents the isotonic saline infusion (o—o = isotonic saline).

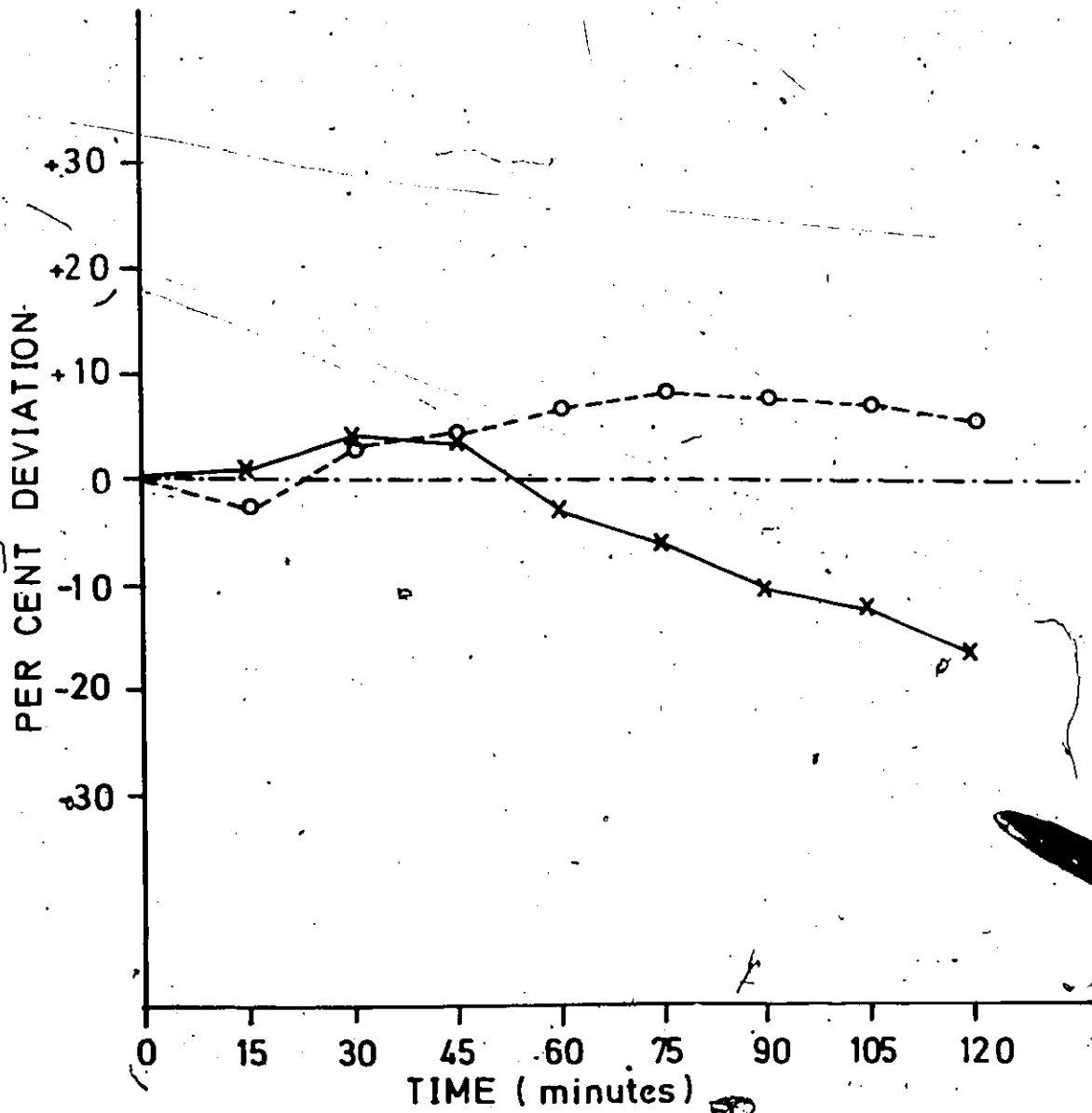
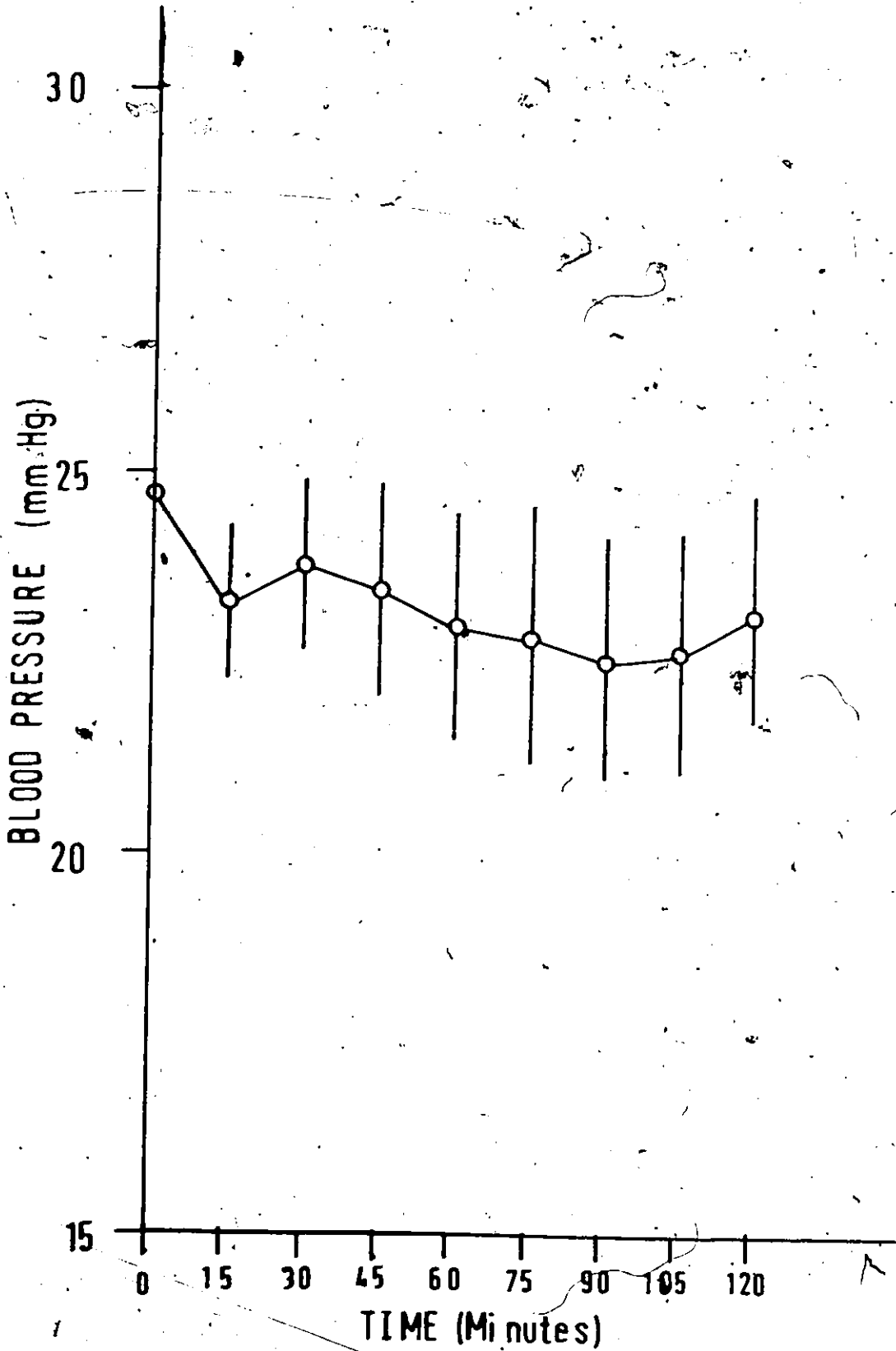


FIGURE 8

The effect of infusing calcium gluconate solution on blood pressure in eels twenty-four hours after Stanniectomy.

Values are means \pm S.E.M.



3. EFFECT OF INDUCED HYPOCALCEMIA ON BLOOD PRESSURE IN EELS

A) CONTROL GROUP

The effects of the double cannulation procedure and a distilled water environment on plasma calcium levels and blood pressure are summarized in Table XVI. Total plasma calcium levels remained stable during the first two-hour recording period (Period A-Table XVI) but decreased slightly during the second two-hours (non-significant $P > 0.10$) (Period B-Table XVI). On the other hand, plasma ionic calcium values increased significantly ($P < 0.05$) over the four-hour recording period but the magnitude of this increase was much lower than that observed in two-week Stanniectomized eels. Blood pressure fluctuated slightly but did not change significantly ($P > 0.10$) during the recording period (Table XVI) despite the overall drop of 2 mm of Hg (Figure 9).

B) EFFECT OF EDTA INFUSION IN INTACT FISH ON PLASMA IONIC CALCIUM AND BLOOD PRESSURE

Data indicating the effect of EDTA infusion on plasma ionic calcium levels and blood pressure are provided in Table XVII. Plasma ionic calcium levels decreased significantly ($P < 0.05$) but the decrease in blood pressure proved to be non-significant ($P > 0.10$). Nevertheless, there was a tendency for blood pressure to drop during the EDTA infusion (Figure 10).

During the period of EDTA infusion the blood pressure showed an initial decline from T+0 min. until T+60 min.. A small

TABLE XVI

EFFECT OF DOUBLE CANNULATION PROCEDURE ON PLASMA CALCIUM AND BLOOD PRESSURE
 IN TAP-WATER ADAPTED INTACT EELS TESTED IN DISTILLED WATER ^a

PERIOD	TOTAL PLASMA CALCIUM AT T + 0 min. mEq/l	TOTAL PLASMA CALCIUM AT T + 120 min. mEq/l	PLASMA IONIC CALCIUM AT T + 0 min. mEq/l	PLASMA IONIC CALCIUM AT T + 120 min. mEq/l	BLOOD PRESSURE AT T + 0 min. mm Hg	BLOOD PRESSURE AT T + 120 min. mm Hg
PERIOD A	4.61 ± 0.12 (9)	4.61 ± 0.11 (9)	1.28 ± 0.07 (9)	1.48 ± 0.05 (9)	27.56 ± 1.88 (9)	25.88 ± 2.02 (9)
PERIOD B	4.61 ± 0.11 (9)	4.46 ± 0.09 (9)	1.48 ± 0.05 (9)	2.38 ± 0.24 (9)	23.22 ± 1.98 (9)	25.22 ± 1.68 (9)

a values are means ± S.E.M (N)

b P < 0.05 comparisons between parameters made in horizontal columns

c P < 0.05 comparisons between parameters made in horizontal columns

d Period A corresponds to the first two hours and Period B corresponds to the second two hours

FIGURE 9

The effect of the double cannulation procedure and four hours of blood pressure recording on intact eels in a distilled water environment.

The break in the line at $T = 120$ min. represents the one milliliter blood sample which was taken at the end of the first two hour recording period (Period A). The second two hour recording period (Period B) begins at $T + 120$ min on the graph for the sake of convenience although there was a 10 minute break while the blood sample was taken. Values are means \pm S.E.M.

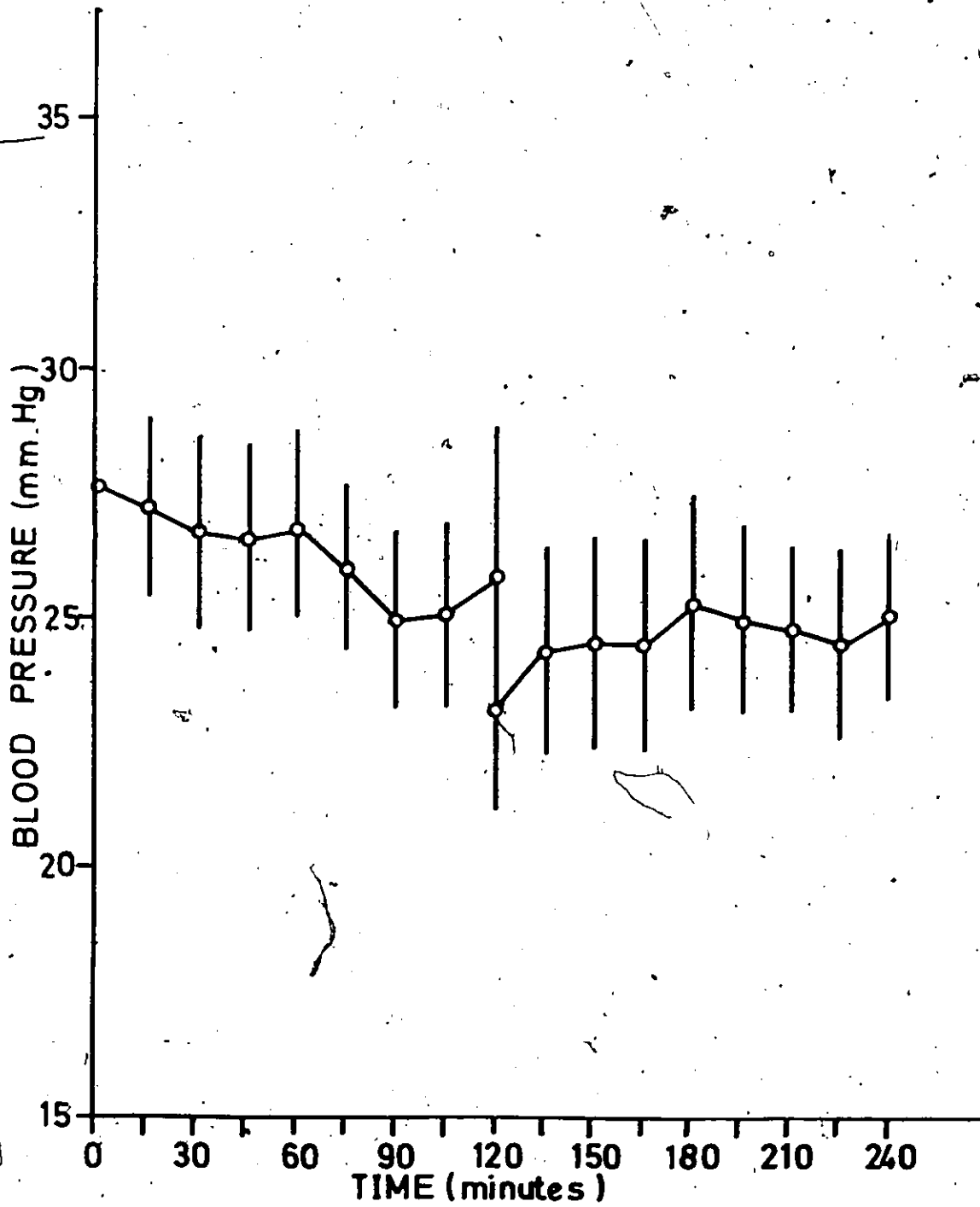


TABLE XVII
EFFECT OF EDTA INFUSION ON PLASMA IONIC CALCIUM AND BLOOD PRESSURE IN

INTACT EELS^a

TIME PERIOD	PLASMA IONIC CALCIUM mEq/l	EDTA INFUSION	BLOOD PRESSURE (mm Hg)	NaCl INFUSION
Initial reading	3.21 ± 0.2 (8)	28.44 ± 2.68 (8)	25.13 ± 2.25 (8)	
After two hours	2.27 ± 0.16 (8) ^b	24.63 ± 2.28 (8)	23.07 ± 2.44 (8)	

^a all values are means ± S.E.M. (N)

^b P < 0.05 compared to initial recording

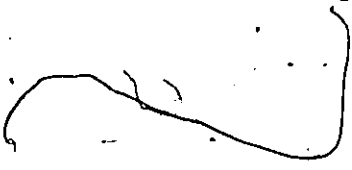
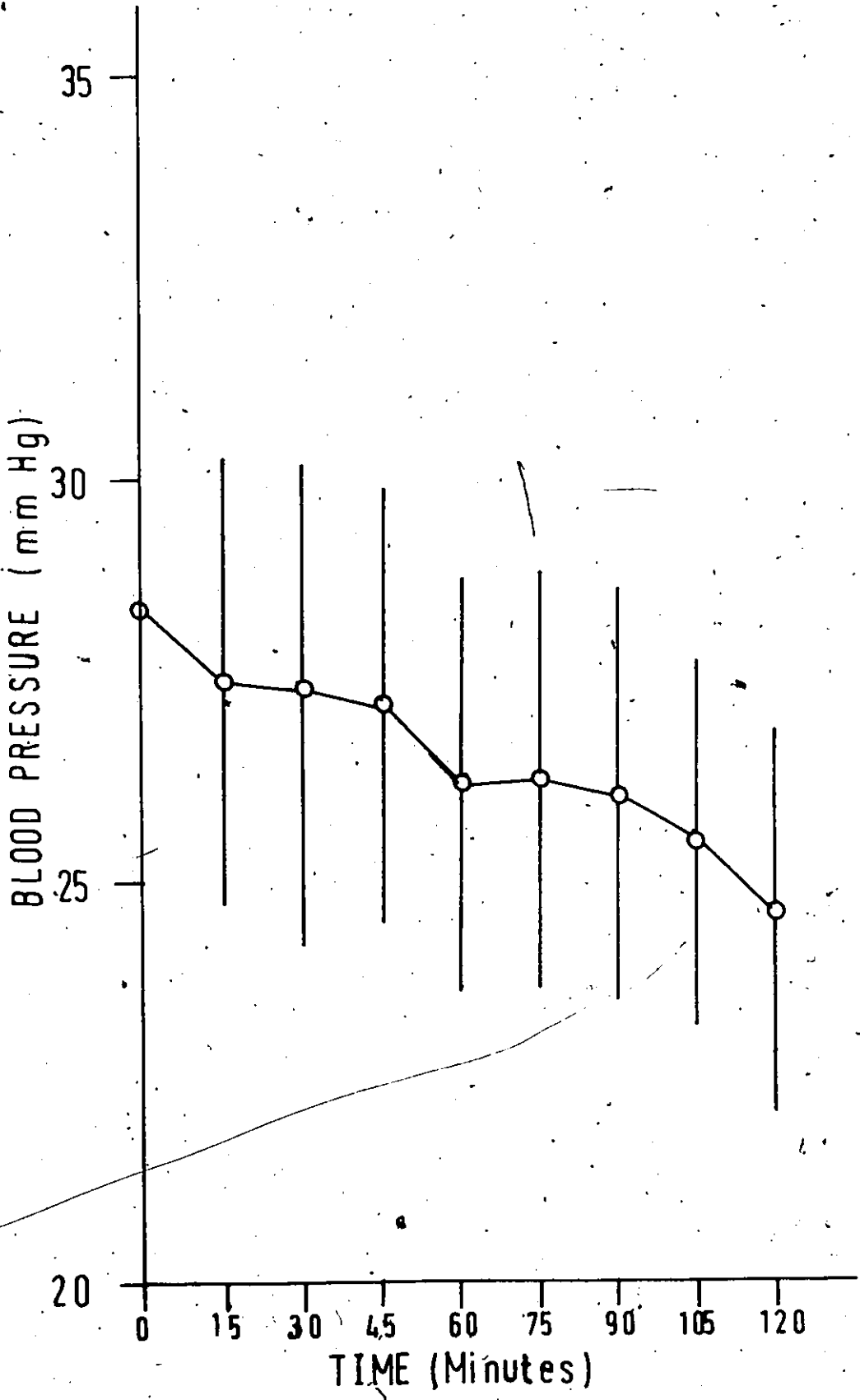


FIGURE 10

The effect of infusing a tetra-sodium EDTA solution on blood pressure in intact eels in a distilled water environment.

The values are means \pm S.E.M.



peak was observed at T+75 min. after which blood pressure decreased until the end of the infusion period (T+120 min). The infusion of isotonic saline resulted in a non-significant ($P>0.10$) decrease in blood pressure (Table XVII).

c) EFFECT OF SODIUM CITRATE INFUSION IN INTACT FISH ON PLASMA IONIC CALCIUM LEVELS AND BLOOD PRESSURE

Plasma ionic calcium showed a significant ($P<0.05$) decrease as a result of sodium citrate infusion (Table XVIII). Blood pressure again failed to decrease significantly ($P>0.10$) (Table XVIII) but the overall trend was a reduction in blood pressure (Figure 11).

Blood pressure fluctuated considerably during the period of citrate infusion. From T+0 min. until T+60 min. blood pressure alternately decreased and increased, but after T+60 min. blood pressure showed a steady decline until the end of the infusion period (T+120 min). Isotonic saline infusion resulted in a slight non-significant ($P>0.10$) decrease in blood pressure (Table XVIII).

4. EFFECT OF HYPOCALCEMIA AND HYPERCALCEMIA ON BLOOD PRESSURE IN INTACT FISH

Table XIX summarizes the data obtained when a period of citrate infusion was followed by a period of calcium infusion. Total plasma calcium levels show no significant change ($P>0.10$) but plasma ionic calcium levels decreased as a result of citrate infusion. The

TABLE XVIII

EFFECT OF SODIUM CITRATE/INFUSION ON PLASMA IONIC CALCIUM AND BLOOD PRESSURE IN INTACT EELS ^a

TIME PERIOD	PLASMA IONIC CALCIUM mEq/l	CALCIUM INFUSION	BLOOD PRESSURE (mm Hg)	NaCl INFUSION
Initial reading.	3.50 ± 0.35 (6)	29.33 ± 3.83 (6)	22.75 ± 3.64 (6)	
After two hours of infusion	1.83 ± 0.31 (6) ^b	25.33 ± 3.83 (6)	21.33 ± 3.57 (6)	

^a values are means ± S.E.M. (N)

^b P < 0.05 compared to initial recording

FIGURE 11

The effect of infusing a sodium citrate solution on blood pressure in intact eels in a distilled water environment.

Values are means \pm S.E.M.

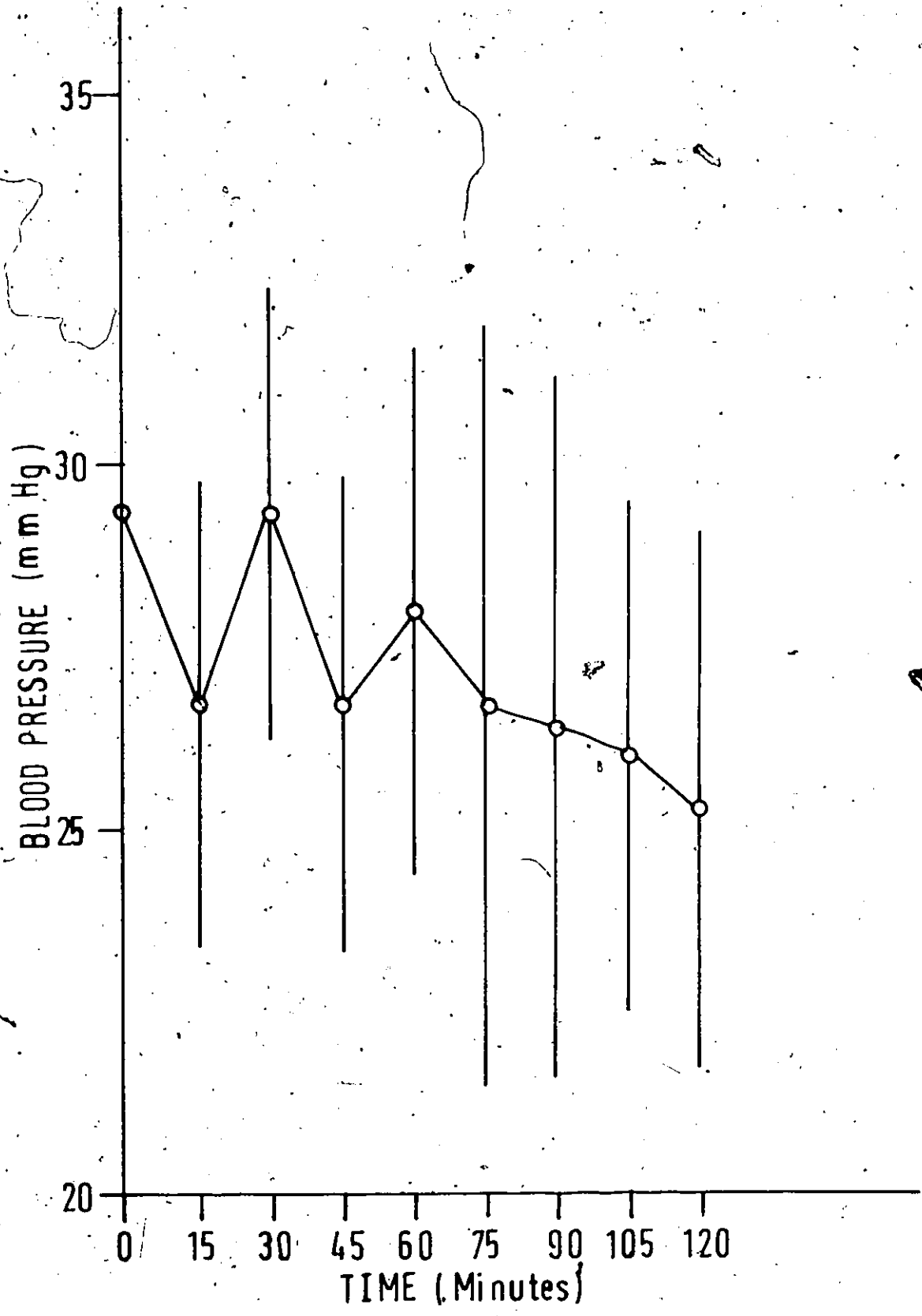


TABLE XIX

EFFECT OF CITRATE AND CALCIUM CHLORIDE INFUSION ON PLASMA CALCIUM AND BLOOD PRESSURE IN INTACT EELS ^a

TREATMENT	TOTAL PLASMA CALCIUM AT T + 0 min. ^b mEq/l	TOTAL PLASMA CALCIUM AT T + 120 min. ^b mEq/l	PLASMA IONIC CALCIUM AT T + 0 min. ^b mEq/l	PLASMA IONIC CALCIUM AT T + 120 min. ^b mEq/l	BLOOD PRESSURE AT T + 0 min. ^b mm Hg	BLOOD PRESSURE AT T + 120 min. ^b mm Hg
CITRATE INFUSION	6.08 ± 0.21 (8)	5.83 ± 0.16 (8)	1.55 ± 0.10 (8)	Less than 1.0 (8)	23.88 ± 1.04 (8)	18.0 ± 3.49 (8)
CALCIUM CHLORIDE INFUSION	5.83 ± 0.16 (8)	12.21 ± 0.22 (8)	Less than 1.0 (8)	2.22 ± 0.22 (8)	16.0 ± 2.99 (8)	20.75 ± 1.71 (8)

^a values are means ± S.E.M. (N)

^b P < 0.05
^c P < 0.05
^d P < 0.05

comparisons made in horizontal columns

amount of decrease could not be determined since the potentials generated by the plasma samples in the calcium electrode were much lower than those given by the least concentrated standard. Blood pressure also showed a significant decrease ($P < 0.05$).

The effect of calcium chloride infusion was markedly dissimilar. Total plasma calcium levels increased significantly ($P > 0.01$), plasma ionic calcium levels showed an increase from the previously undetectable level to 2.22 ± 0.22 meq/l and blood pressure showed a significant increase ($P < 0.05$).

During the period of citrate infusion (Figure 12) blood pressure decreased until T+30 min. at which time an increase was noted until T+60 min. Blood pressure then declined steadily until T+105 min. at which point a small increase was noted. At T+120 min., citrate infusion ceased and calcium chloride infusion began. Calcium chloride (Figure 12) infusion was characterized by a steady increase until T+210 min. at which point a slight decrease in blood pressure was observed. This was followed by another increase until T+240 min. which was the end of the infusion period.

5. MISCELLANEOUS OBSERVATIONS

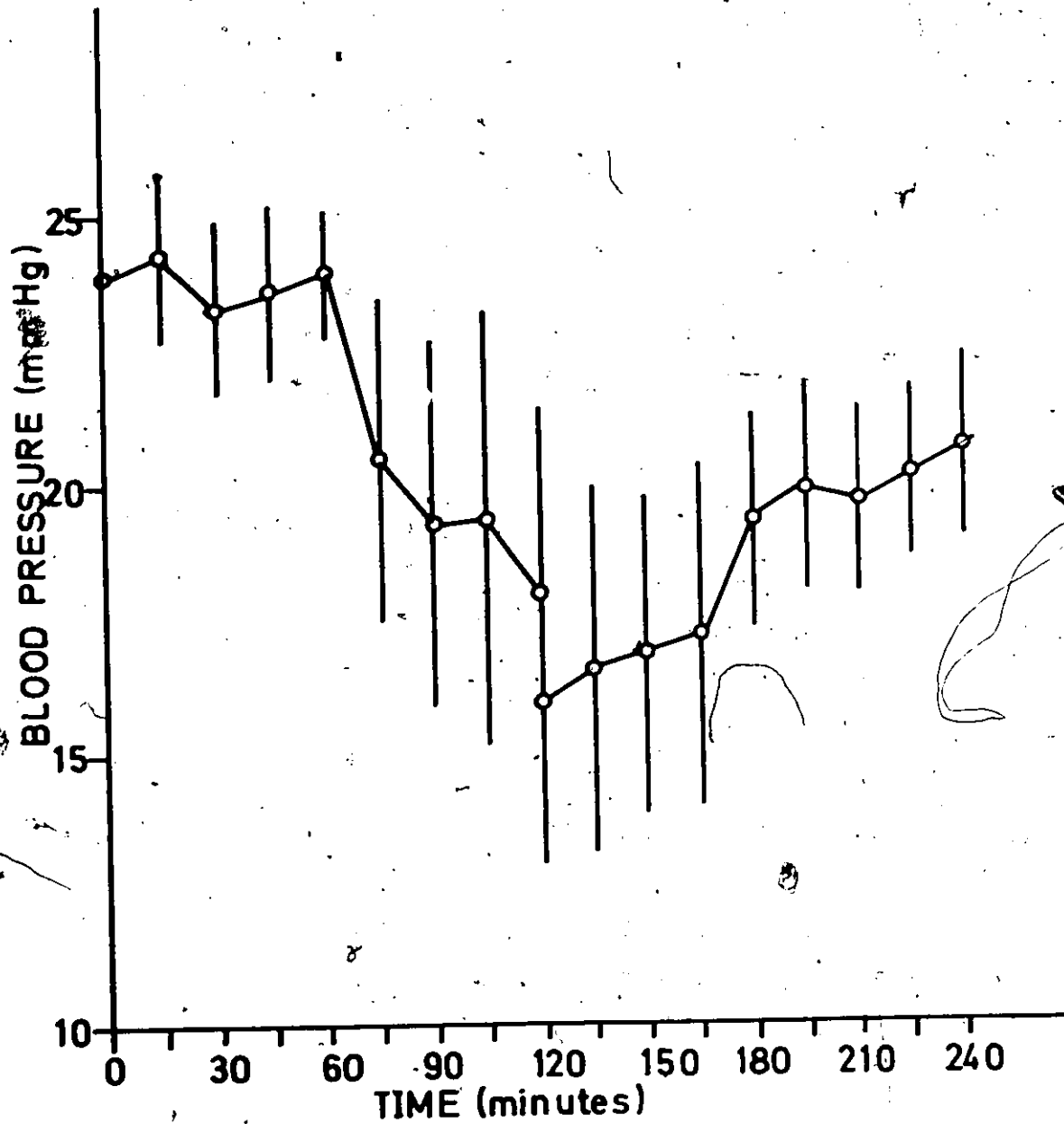
A) EFFECT OF ANGIOTENSIN II AND CORPUSCULAR EXTRACT ON PLASMA CALCIUM LEVELS IN INTACT FISH.

One hour after the injection of Angiotensin II a slight but non-significant ($P > 0.10$) decrease in total plasma levels and a significant ($P < 0.05$) increase in plasma ionic calcium levels were

FIGURE 12

The effect of infusing a sodium citrate solution followed by a calcium chloride solution on blood pressure in intact eels in a distilled water environment.

Values are means \pm S.E.M. The break in the line at T + 120 min. represents the time at which a one milliliter blood sample was taken after the period of sodium citrate infusion. The period of calcium chloride infusion begins at T + 120 min. on the graph despite the fact that there was a break of approximately 10 minutes, required to change the infusion solutions and take the blood sample.



observed (Table XX). Corpuscular extract resulted in a significant ($P < 0.05$) decrease in plasma ionic calcium levels and a non-significant increase ($P > 0.10$) in total plasma calcium one hour after injection (Table XX).

3) EFFECT OF ANGIOTENSIN II AND CORPUSCULAR EXTRACT
INJECTION ON BLOOD PRESSURE IN INTACT FISH.

Shown in Table XXI are the blood pressures before injection and one hour after injection of the test solutions. Injection of Angiotensin II resulted in a slight non-significant ($P > 0.01$) decrease in blood pressure after one hour. Blood pressure shows a non-significant ($P > 0.10$) increase one hour after the injection of corpuscular extract. Similar results were obtained with the isotonic saline injection. One hour after this injection a slight increase in blood pressure was observed which was not found to be significant ($P > 0.10$).

Figure 13 indicates that the response of blood pressure to Angiotensin II consists of a monophasic hypertensive response with the peak blood pressure recorded five minutes (T+5 min.) after injection. Blood pressure began to decline but was still higher than the initial recording at T+15 min. At T+20 min. blood pressure had fallen below the initial pressure and continued to decrease until T+30 min. At this point, a gradual increase was observed but the blood pressure had not returned to the initial level at T+60 min. which was the end of the recording period. This was in direct contrast with the data obtained as a result of injection of a saline extract

TABLE XX
EFFECT OF ANGIOTENSIN II AND CORPUSCULAR EXTRACT ON PLASMA CALCIUM
LEVELS IN INTACT EELS^a

TREATMENT	TOTAL PLASMA CALCIUM AT T + 0 min. mEq/l	TOTAL PLASMA CALCIUM AT T + 60 min. mEq/l	PLASMA IONIC CALCIUM AT T + 0 min. mEq/l	PLASMA IONIC CALCIUM AT T + 60 min. mEq/l
Angiotensin II Injection	4.53 ± 0.32 (6)	4.14 ± 0.03 (6)	2.50 ± 0.34 (6)	3.52 ± 0.30 (6)
Corpuscular extract Injection	4.14 ± 0.03 (6)	4.68 ± 0.19 (6)	3.52 ± 0.30 (6)	1.38 ± 0.12 (6)

a values are means ± S.E.M. (N)
b P < 0.05
c P < 0.05
comparisons made in horizontal columns

TABLE XXI
 BLOOD PRESSURE RESPONSE TO ANGIOTENSIN II, CORPUSCULAR EXTRACT AND
 ISOTONIC SALINE INJECTION IN INTACT EELS^a

TIME PERIOD	TREATMENT		
	ANGIOTENSIN II mm Hg	CORPUSCULAR EXTRACT mm Hg	ISOTONIC SALINE mm Hg
Blood pressure before injection (Time T)	27.4 ± 3.75 (6)	24.6 ± 3.50 (6)	24.6 ± 3.30 (6)
Blood pressure at T + 60 min.	25.8 ± 3.68 (6)	25.60 ± 3.28 (6)	25.8 ± 2.48 (6)

^a values are means ± S. E. M. (N)

FIGURE 13

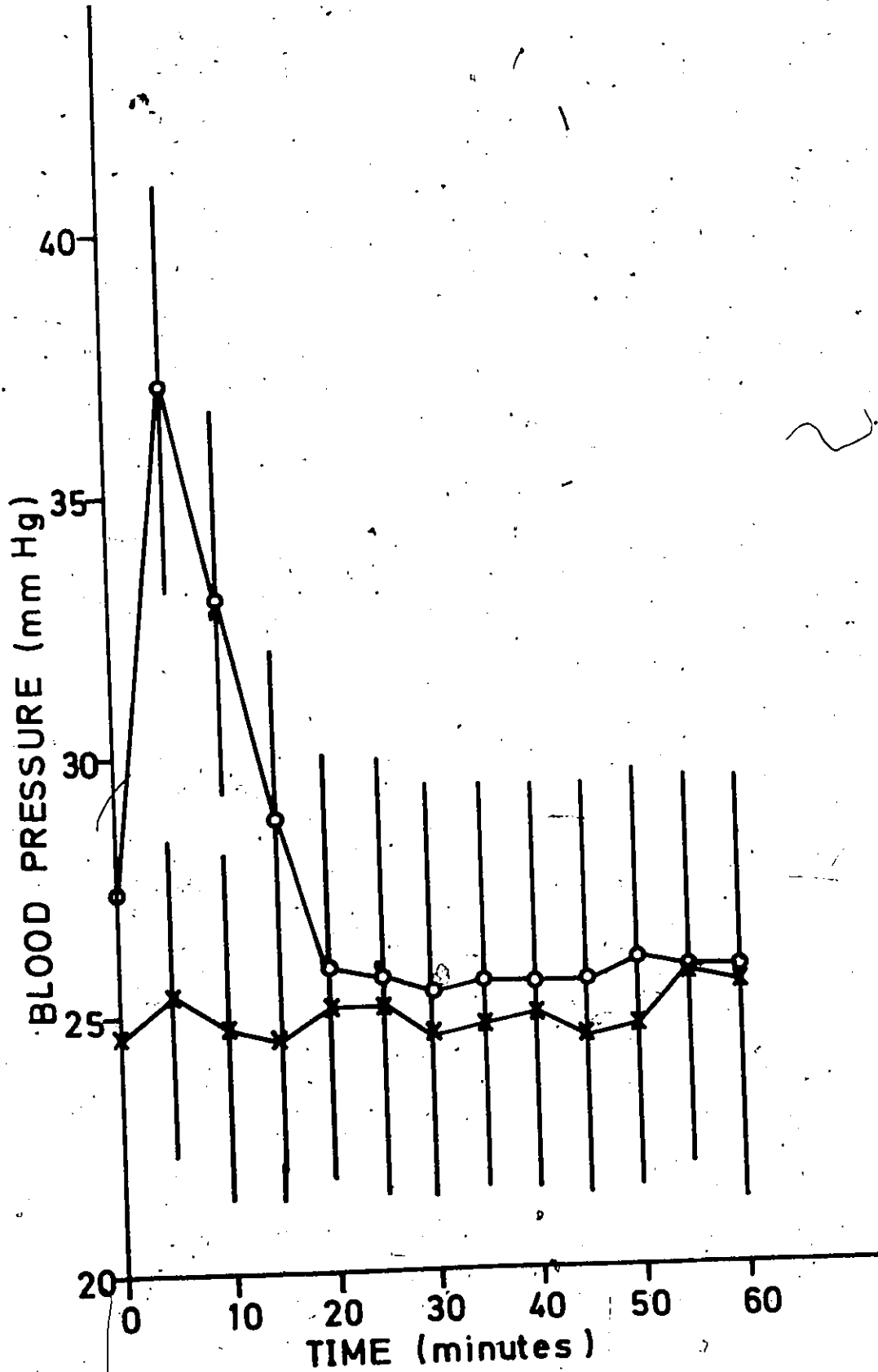
The effect of Angiotensin II injection and corpuscular saline extract injection on blood pressure in intact eels.

LEGEND

—○—○— represents the effect on blood pressure of the Angiotensin II injection

—x—x— represents the effect on blood pressure of the corpuscular saline extract injection.

Values are means \pm S.E.M.



of Corpuscles of Stannius tissue (Figure 13). Whereas Angiotensin II resulted in an initial massive increase in blood pressure, a small increase was observed as a result of corpuscular extract injection. There was no demonstrable pressor effect from corpuscular extract as injection of isotonic saline (Figure 14) resulted in a similar pattern of blood pressure response of the same order of magnitude, as the corpuscular extract.

C) BEHAVIORAL OBSERVATION

Stanniectomized eels adapted to tap-water appeared more inert and asthenic when compared to sham-operated or intact eels adapted to tap-water; they were easier to catch in the dip-nets used for removing eels from the holding tanks. Tap-water adapted Stanniectomized eels also appeared to be more susceptible to anaesthesia than animals of other groups including Stanniectomized eels adapted to an acalcemic water environment.

D) CARDIOVASCULAR PHENOMENA

Tap-water adapted Stanniectomized eels appear to have a slower heart rate than do/sham-operated and intact control eels adapted to tap-water (Table XXII).

FIGURE 14

The effect of isotonic saline injection on blood pressure in intact eels.

Values are means \pm S.E.M.

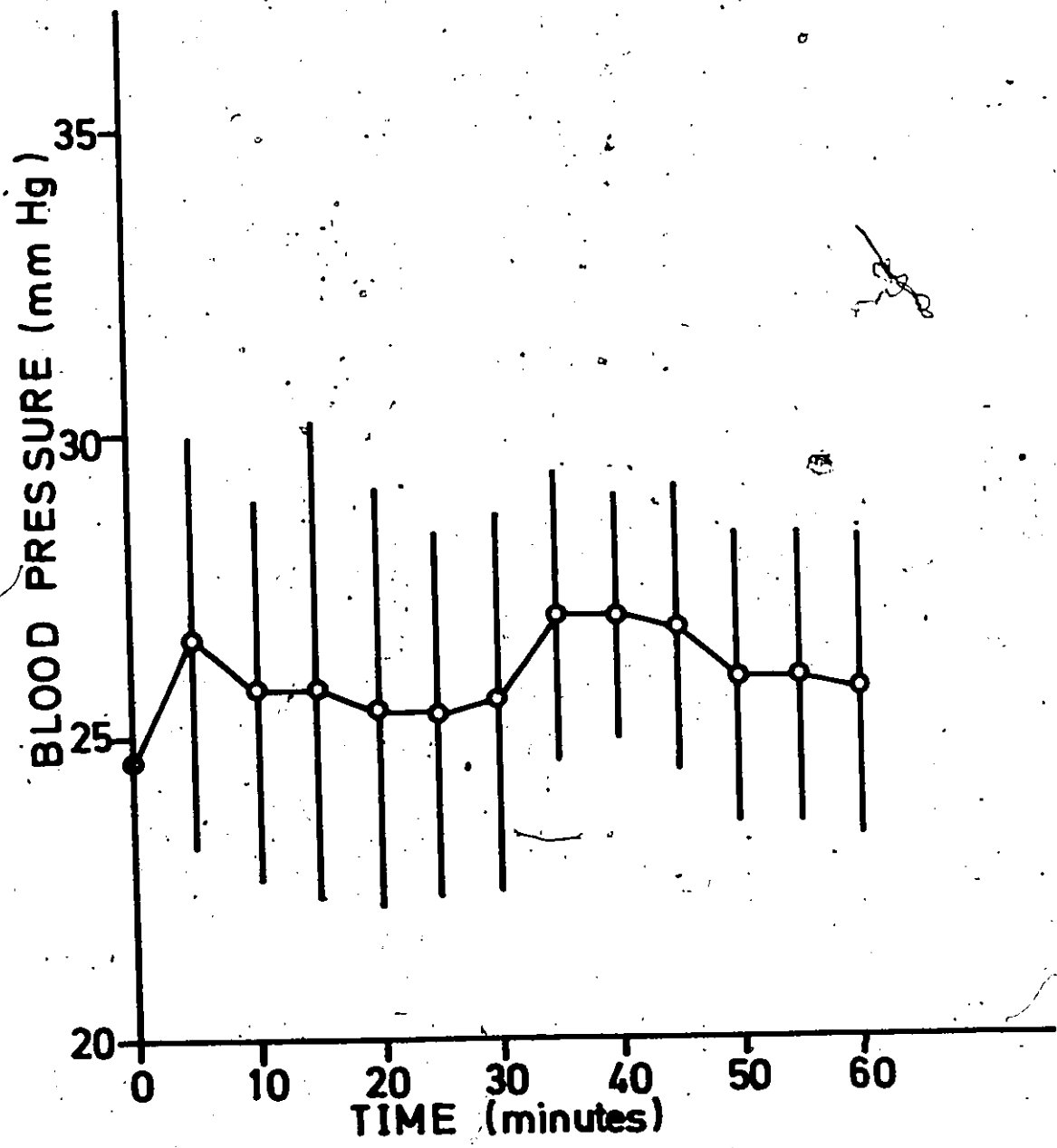


TABLE XXII

HEART RATES OF STANNIECTOMIZED, SHAM-OPERATED AND INTACT
CONTROL EELS ADAPTED TO TAP-WATER^a

GROUP	HEART RATE (Beats/min.).
Stanniectomized	47 ± 2.26 (6)
Sham-Operated	66 ± 6.0 (5)
Intact Controls	90 ± 0 (2)

^aValues are means ± S.E. M(N)

Electrocardiograms of Stanniectomized eels adapted to tap-water show that these animals have a reduced P wave, a reduced QRS component and an inverted T wave when compared with tap-water adapted sham-operated and intact control eels. (Figure 15).

6. STATISTICAL CORRELATIONS

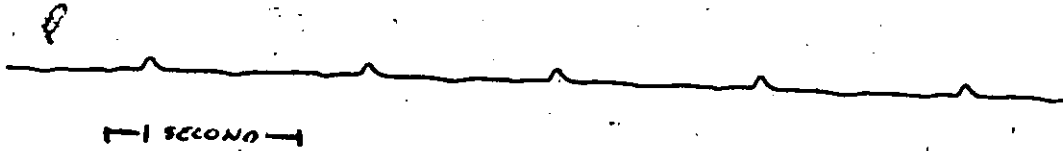
Overall a significant negative ($P < 0.05$) correlation was found between total plasma calcium levels and blood pressure. As plasma calcium increases blood pressure decreases. At total plasma calcium levels which approach those found in Stanniectomized eels, the blood pressure shows a decrease similar to that found in Stanniectomized eels...

It appears that plasma ionic calcium has a more important role than does the bound calcium since alterations in ionic calcium

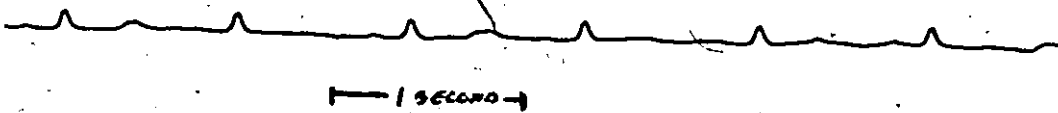
~~FIGURE~~ 15

Electrocardiograms of a four-week Stanniectomized eel, a four-week Sham-operated eel and an Intact control eel.

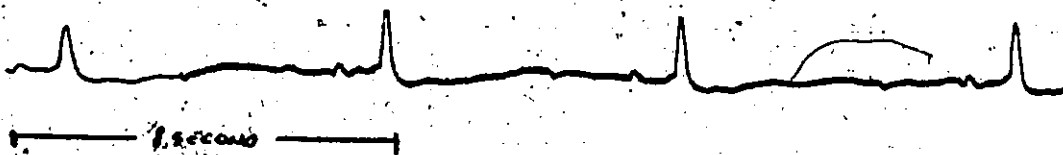
Stanniectomized



Sham-operated



Intact Controls



result in significant changes in blood pressure. Infusion of a chelating agent such as sodium citrate may result in a significant change in plasma ionic calcium and blood pressure without significantly altering total plasma calcium levels. A highly significant ($P < 0.01$) correlation was established between plasma ionic calcium and blood pressure overall. It was found that as plasma ionic calcium levels increase blood pressure decreases.

Plasma ionic calcium levels were found to be approximately 30% of the total plasma calcium. A mean value (\pm S.E.) of 5.31 ± 0.11 meq/l was obtained for total plasma calcium while a mean value (\pm S.E.) of 1.57 ± 0.07 meq/l was obtained for plasma ionic calcium. A highly significant ($P < 0.01$) correlation was established between plasma ionic calcium and total plasma calcium. It was observed that as total plasma calcium increased so did plasma ionic calcium. These values were obtained from intact fish which had not been infused and which were kept in dechlorinated tap-water.

DISCUSSION

1. CALCIUM AND BLOOD PRESSURE - MECHANISMS OF ACTION

The experimental evidence gathered all indicates that any major alteration in plasma calcium levels results in a decrease in systemic blood pressure. It also suggests that the Corpuscles of Stannius do not possess a pressor substance of any physiological importance but that the decrease in blood pressure following Stanniectomy is a result of disturbed calcium homeostasis.

There are several possible modes of action by which calcium could result in a decrease in blood pressure:

1. an effect on the production of corticosteroids
2. an inhibitory effect on renin release or action
3. an effect on the cardiovascular system

A) CALCIUM AND CORTICOSTEROID PRODUCTION

Calcium is required for the biosynthesis of cortisol and for the effect of ACTH on interrenal tissue (Farese, 1971b). It is also required for the stimulatory effect of cyclic AMP on the interrenal tissue (Farese, 1971a). In addition, calcium seems to stimulate adrenal protein synthesis which, in turn, is involved in the steroidogenic action of the interrenal tissue (Farese, 1971b). In the eel, Anguilla anguilla, cortisol appears to control salt and water excretion (Chan, Rankin and Chester Jones, 1969). In mammals, cortisol has been observed to cause an increase in systemic blood pressure. Conversely, adrenal insufficiency results in a fall in blood pressure (Friedman, 1965).

Cortisol biosynthesis in the eel appears to be affected by high calcium levels. This is supported by both in vivo and in vitro evidence. In vitro experiments have shown that calcium is required for cortisol biosynthesis and for a stimulatory effect of ACTH (Leloup-Hatey, 1970a). However, high or low calcium concentrations have an inhibitory effect on cortisol biosynthesis; the high inhibitory level of calcium being of the same order of magnitude as the plasma calcium levels found in two-week tap-water adapted Stanniectomized eels (Leloup-Hatey, 1970a). Whilst Stanniectomy results in persistent hypertrophy of the interrenal tissue, after approximately two weeks plasma cortisol levels decline below those values found in intact fish (Leloup-Hatey, 1970b; Fenwick and Forster, 1972). The hypertrophy of the interrenal tissue is explained by the fact that increases in plasma calcium result in an increase in adrenal cortisol secretory rates (Marotta and Lau, 1970), until the level at which cortisol biosynthesis is inhibited is reached (Leloup-Hatey, 1970a). Also, a fall in plasma sodium levels is observed in Stanniectomized eels (Fontaine, 1964). This hyponatremia may be explained by a lack of cortisol, or by increased plasma concentrations of calcitonin which is known to have a natriuretic effect in mammals when present in high concentrations (Copp, 1969). Thus since adrenal insufficiency could result in a decrease in blood pressure in eels, high plasma calcium levels which tend to inhibit cortisol biosynthesis could account for the decrease in blood pressure following Stanniectomy.

Plasma calcium levels obtained after calcium infusion

and as a result of Stanniectomy were indeed high enough to result in a decrease in cortisol biosynthesis. The high inhibitory level was found to be approximately 5.0 mM/l (Leloup-Hatey, 1970a). Plasma calcium levels after calcium infusion ranged from 5.0 mM/l to 8.0 mM/l (Table XII) which are certainly above the level required for inhibition of cortisol biosynthesis. Plasma calcium levels in tap-water adapted two-week Stanniectomized eels were also above this level being in the order of 5.1 mM/l (Table V). Thus, one possible mechanism of action of calcium is an inhibition of cortisol biosynthesis which could result in a decrease in blood pressure.

However, there is evidence which tends to disagree with this hypothesis. Recent work has shown that plasma cortisol may have a half-life in the eel blood plasma of up to six hours (Ball et al, 1971). Therefore, no decline in blood pressure would be observed in a two-hour period of calcium infusion. But a significant decrease in blood pressure was observed (Table XII) when plasma calcium levels approached those of Stanniectomized eels at the end of the two-hour infusion period. This would preclude the possibility that lack of cortisol is causing a decrease in blood pressure since there is no evidence that calcium levels impair the function of pre-existing steroids in the blood. It has also been found that acute increases in plasma calcium levels tend to increase adrenocortical secretory rates (Marotta and Lau, 1970). This evidence argues against the hypothesis that increases in plasma calcium obtained as a result of calcium infusion are causing a decreased rate of cortisol biosynthesis.

If plasma sodium levels may be taken as an indication of cortisol function then another argument against the hypothesis is provided. The pattern of decreased plasma sodium levels has been observed in both the tap-water Stanniectomized group and the acalcemic water Stanniectomized group (Appendix-Table I). This would indicate an impairment of cortisol biosynthesis or release. No decline in systemic blood pressure and no increase in plasma calcium levels were observed in the acalcemic water Stanniectomized group. This suggests that the decrease in blood pressure is not caused by a fall in plasma cortisol levels but rather through some other agency.

Blood pressure was also observed to decline when the fish were infused with sodium citrate (Tables XVIII and XIX). It is doubtful that cortisol biosynthesis is inhibited in this case since it has been found that hypocalcemia has no discernible effect on adrenocortisol secretory rates and that corticosteroidogenesis requires only minute amounts of calcium (Marotta and Lau, 1970). Frank inhibition of cortisol biosynthesis of eel interrenal homogenates in vitro was only observed when there was no calcium present in the incubation medium (Leloup-Hatey, 1970a). Thus, it appears unlikely that cortisol biosynthesis is inhibited during periods of hypocalcemia and that the decline in blood pressure observed is not attributable to a lack of control.

B) CALCIUM AND RENIN RELEASE

The rate of renin production and release appears to be controlled by several factors. Decreased systemic blood pressure,

decreased renal perfusion pressure, the sympathetic nervous system and catecholamines all appear to act in a stimulatory fashion (Michelakis, 1971b; Waugh, 1972). Experimental evidence indicates that angiotensin II inhibits renin release from the kidney in a short-loop negative feed-back system (Michelakis, 1971a).

Calcium and sodium tend to affect the rate of renin release from the kidney in vitro. In the dog kidney the rate of renin release tends to decrease as sodium concentrations increase (Michelakis, 1971a). Increases in plasma calcium cause an increase in the rate of renin release but when calcium concentrations are greater than 2.5 mM/l renin release is inhibited (op. cit., 1971a). Since plasma calcium levels are much higher than this following Stanniectomy (approximately 5.0 mM/l, Table V) it is very possible that renin release from the eel kidney was inhibited. This inhibition of renin release could then result in a decrease in systemic blood pressure.

At the present time little is known about the effects of hypocalcemia on renin release. In vitro experiments have shown that when calcium is absent from the incubation medium renin release from the kidney is inhibited (Michelakis, 1971a). It is very possible that hypocalcemia could result in a decrease in renin release since it appears that calcium is necessary for renin release from the kidney in vitro. Hypocalcemia would then result in a decrease in blood pressure.

Thus the second possible mechanism of action of calcium

could be an inhibition of the rate of renin release which would lead to a decrease in blood pressure. However, this mechanism fails to explain why blood pressure does not show a significant decrease when plasma calcium levels are in the range of 3.0 mM/l to 4.0 mM/l. The calcium inhibitory level was found to be 2.5 mM/l and increases above this level should result in a decrease in blood pressure. But eels infused with calcium gluconate (Tables IX and XV) do not show a significant decrease in blood pressure despite the fact that the plasma calcium levels resulting from this infusion were above the 2.5 mM/l inhibitory level. It is possible that eel renin is less sensitive to calcium inhibition than is dog renin. This would explain why blood pressure does not show a significant decrease in the 3.0 mM/l to 4.0 mM/l range. However, there is no evidence to support this purely speculative hypothesis. In any event it seems unlikely that the decrease in blood pressure following Stanniectomy results from a decrease in the rate of renin release from the kidney of the eel.

C) CALCIUM AND THE CARDIOVASCULAR SYSTEM

Blood pressure is controlled to a large extent by the cardiovascular system without any extrinsic influences. Any disturbance in the functioning of the cardiovascular system would result in a disturbance in blood pressure. This system is composed of two types of muscle; cardiac and smooth muscle. These both depend on the presence of calcium for contraction and relaxation as does striated

(skeletal) muscle. Disturbances in extra-cellular calcium tend to cause a change in the contractility of both the cardiac muscle fibers of the heart and the smooth muscle cells of the blood vessels.

Hypercalcemia appears to cause a reduction in heart rate (Marshall, 1961). This effect is brought about by two separate actions of calcium on the muscle fibers themselves. High concentrations of calcium result in a hyperpolarization of excitable membranes as calcium and sodium compete for reception sites on the membranes (Dubois and Bergman, 1971). These membranes have been found to have a greater affinity for calcium ions than for sodium ions (op.cit., 1971). This would result in an inhibition of the propagation of an action potential. Since the propagation of the action potential is inhibited by high calcium levels this would result in a decrease in heart rate, a decrease in cardiac output and subsequently, a decrease in blood pressure. In addition, high calcium concentrations tend to inhibit norepinephrine binding in the cardiac muscle (Balasubramanian and Dhalla, 1971; Nash, Tu and Martin, 1971). This action would also tend to decrease heart rate. Indeed, Stanniectomized eels appear to have a slower heart rate than do intact eels (47 beats per minute as compared to 90 beats per minute (Results, Table XXII)). Electrocardiograms recorded from Stanniectomized eels show disturbances in the electrical properties of the heart. Stanniectomized eels have a much reduced P wave and QRS component and also show an inverted T wave (Figure XV). High calcium concentrations tend to enhance the muscarinic action of acetylcholine in mammals, i.e. a decrease in

heart rate and a drop in blood pressure by the action of acetylcholine on the muscarinic receptors (Friedman et al, 1967).

Although the electrical activity of the heart is decreased by high calcium concentrations, the actual mechanical process of contraction may be increased (Benmouyal, 1971). High calcium concentrations appear to increase glucose metabolism and force of contraction in isolated perfused guinea pig hearts (op.cit., 1971). However, an increase in force of contraction brought about by such factors as stretch or an increase in electrical stimulation does not affect the intracellular distribution of calcium (Tomlinson and Dhalla, 1972). In rats, it appears that the contractile elements are buffered against massive increases in extra-cellular calcium by the mitochondria which can pick up and store large amounts of calcium (Ueba, Ito and Chidsey, 1971). This action prevents calcium being picked up by the microsomal fraction, thus affecting the contractile elements of the heart muscle although serious conduction abnormalities are found at high extra-cellular calcium levels (op.cit., 1971). The overall effect of an increase in extra-cellular calcium is thus a decrease in the electrical activity of the heart while the contractile elements remain largely unaffected. This results in a decrease in heart rate which in turn would lead to a decrease in blood pressure. The high plasma calcium levels obtained as a result of Stanniectomy or calcium chloride infusion may affect the heart rate of eels in a similar fashion, resulting in a decrease in blood pressure. In vitro studies of rat hearts have shown that the most

severe effects are noted at calcium levels above 4.5 mM/l (Ueba, Ito and Chidsey, 1971). If the eel heart displayed the same tolerance for calcium as the isolated rat heart, this would explain the apparent lack of response of plasma calcium levels in the 3.0 - 4.0 mM/l range.

Hypocalcemia also results in a disturbance in heart rate. Contractility is decreased and the duration of the action potential is increased (Brady and Tan, 1966). This would result in a decrease in force of contraction, a decrease in heart rate and a decrease in cardiac output which in turn would result in a decrease in blood pressure. This would explain the decrease in blood pressure obtained when the eels were infused with sodium citrate (Table XV). Infusion with EDTA would not give a decrease in blood pressure until plasma ionic calcium is very low since EDTA distorts the cellular membrane of cardiac muscle in such a way as to make it more permeable to calcium (Winegrad, 1971). This would then allow the heart to contract at much lower levels of extra-cellular calcium than would normally be required, and it would explain why there was an apparent lack of blood pressure response in eels infused with EDTA (Table XVII).

Blood pressure is also regulated by the smooth muscle of the blood vessels, particularly the arteries (Guyton, 1971).

Any disturbance in the tonus of the vessel would be reflected as a disturbance in blood pressure. Hypercalcemia results in a loss of contractility in the aortic smooth muscle (Sitrin and Bohr, 1971).

An increase in extra-cellular calcium also results in hyperpolarization of membranes of smooth muscle cells (Axelsson et al, 1967). Hyperpolarization of the membrane would result in a loss of contraction

which in turn would result in a decrease in blood pressure. Thus, an increase in plasma calcium levels would cause a loss of contractility and membrane hyperpolarization in the smooth muscle cells of the blood vessels. This in turn would result in a decreased resistance to blood flow and a decreased resistance to blood pressure. The deformation of membrane structure and increased permeability to calcium produced by EDTA would also explain why it is more difficult to induce a decline in systemic blood pressure by chelating plasma ionic calcium than by increasing plasma calcium levels.

Therefore, hypercalcemia would result in a decrease in blood pressure by its action of slowing the heart and decreasing the contractility of the vascular smooth muscle. Hypocalcemia which also results in a decrease in blood pressure could be affecting both the heart and vascular smooth muscle by causing a loss of contractility in both types of muscle.

Hypercalcemia also has an effect on the nervous system which would ultimately reflect upon the cardiovascular system. Depolarization-evoked transmitter release at motor-endplates is inhibited irreversibly by high calcium concentrations as is spontaneous release of acetylcholine at motor-endplates (Heuser, Katz and Miledi, 1971). This would cause a general failure of any neural response to stress. Stanniectomized eels appear more asthenic and inert than do intact eels at the same temperature. This could be an indication that the nervous system of the Stanniectomized eels is not functioning properly. This is not observed in Stanniectomized.

eels in an acalcemic environment. This is a rather subjective evaluation since no activity tests per se were done on the Stanniectomized eels, but it is substantiated by previous observations on Stanniectomized killifish, Fundulus heteroclitus (Pang, 1971).

Hypocalcemia appears to affect the nervous system of fish. Hypophysectomized Fundulus heteroclitus show tetanic convulsions associated with lowered plasma calcium levels (Pang et al, 1971). These conditions may be corrected by placing these fish in a calcium rich environment (Pang et al, 1971). This would indicate that calcium is necessary for normal nervous conduction since sodium, potassium and chloride remain unaffected by hypophysectomy in these conditions (op.cit., 1971).

Therefore hyper- and hypo-calcemia not only interfere directly with cardiovascular function but may affect it indirectly by action on the nervous system. Both this direct and indirect interference would ultimately result in a decrease in blood pressure.

D) MECHANISM OF ACTION

As it has been seen, high or low calcium levels may affect the cardiovascular system, the nervous system, the rate of renin release from the kidney and to a lesser extent corticosteroid biosynthesis. All of these actions could cumulatively result in a decrease in blood pressure.

It is possible that the high plasma calcium levels which are the result of Stanniectomy may be causing a decline in blood

pressure in the following fashion. As plasma calcium levels increase, the efficiency of the heart as a pump decreases due to the inhibition of action potential propagation by the high levels of extra-cellular calcium. This would result in a decrease in heart rate, a decreased cardiac output and a consequent lowering of blood pressure. Contractility of the cardiac muscle does not appear to be affected to any great extent but it may be slightly decreased. This would result in a loss of force of contraction and a decrease in blood pressure. High calcium levels would also result in a decrease in tonus of the smooth muscle of the blood vessels, which would result in a decrease in peripheral resistance to flow and a drop in blood pressure. Venous return would also decrease which in turn could nullify an effect due to increased loading of the heart that is a consequence of a slower heart rate.

If eels possess a cardioaccelerator center as do mammals, any action of this center would be inhibited by the high calcium levels since high extra-cellular calcium results in a decrease of depolarization induced transmitter release at end-plates and indeed conduction along the axon itself. If renin release by the eel kidney is inhibited by high calcium levels as is renin release by the dog kidney, the renin-angiotensin system would be unable to play any role in the maintenance of blood pressure. Also the release of catecholamines from the sympathetic system is inhibited by high calcium levels since the nervous system itself is inhibited. If catecholamines were released from the interrenal chromaffin tissue, it is very likely

that these would have little effect on blood pressure since high calcium levels tend to inhibit norepinephrine binding and transport in the cardiac muscle.

Therefore, the proposed mechanism is an inhibition of cardiovascular efficiency, i.e. decrease in heart rate and a possible decrease in force of contraction resulting in a decrease in cardiac output, a loss of tonus of the blood vessels and a subsequent reduction in peripheral resistance, all of which result in a decrease in blood pressure. Any extrinsic factors which could possibly overcome this inhibition, such as a cardioaccelerator center, an increase in catecholamine release or the renin-angiotensin system, are themselves inhibited. The role of cortisol in blood pressure maintenance is obscure but if cortisol does play a role in blood pressure maintenance, this would be nullified by the fact that cortisol biosynthesis is inhibited by high calcium levels.

Hypocalcemia also appears to cause a decrease in blood pressure although very low levels of plasma ionic calcium are required to result in a significant decrease in blood pressure. The mechanism of action is probably similar to the effect of hypercalcemia, i.e. a loss of efficiency of the cardiovascular system. However, this appears due to a loss of contractility rather than an inhibition of the electrical activity of the heart and blood vessels. The effect of hypocalcemia on renin release and cortisol biosynthesis remains obscure.

2. THE CORPUSCLES OF STANNIUS AND THE JUXTAGLOMERULAR SYSTEM

Previous work has shown that a saline extract of the Corpuscles of Stannius exhibits potent pressor activity in the eel (Chester Jones et al, 1966). The data obtained as a result of injecting an amount of extract approximately equal to one corpuscle per kilogram body weight do not agree with these published findings. Injection of angiotensin II resulted in an immediate massive increase in blood pressure (approximately 15 mm of Hg increase) but injection of corpuscular extract showed no similar increase even after one hour. Also angiotensin II resulted in an increase in plasma ionic calcium after one hour while the corpuscular extract resulted in a decrease in plasma ionic calcium after one hour. These data suggest that the mode of action of angiotensin II and corpuscular extract are different. Also, in the previous work by Chester Jones et al, (1966), a dose equivalent to two corpuscles per kilogram of body weight was given. This was quite possibly an extra-physiological dose and therefore could perhaps be resulting in effects not present at a physiological level. More recent work has shown that at lower doses the extract of the corpuscles show no pressor activity but larger doses have a pressor effect (Chester Jones et al, 1969).

The juxtaglomerular cells of the kidney have been shown to be the source of renin (Hartroft et al, 1964; Pitcock et al, 1959; Tobian et al, 1959) which is the enzyme responsible for the formation of active angiotensin. These cells appear to be modified smooth muscle cells containing granules which are homogenous, dense and

osmophilic (Barajas and Latta, 1967). These granules bear a resemblance to the β -cell granules of the pancreatic islets (op.cit., 1967). The juxtaglomerular (J.G.) cellular granules stain specifically with Bowie's stain and weakly with aldehyde-fuchsin (Krishnamurthy and Bern, 1969). Periodic acid-Schiff (PAS) stains all granules of the J.G. cells (op.cit., 1969).

The granules of the cells of the Corpuscles of Stannius stain specifically with aldehyde-fuchsin but weakly with Bowie's stain (op.cit., 1969). Also, PAS is observed to stain only some of the granules in a few species (op.cit., 1969). This would indicate that there is a biochemical difference between the J.G. granules and the corpuscular granules.

Despite the differences in staining reactions the corpuscular granules resemble mammalian renin in so far as they are destroyed by acidification to pH 2.0, are non-diffusible through cellophane and are heat-labile (Chester Jones et al, 1966) all of which are properties of mammalian renin (Skeggs et al, 1967).

However, eel corpuscular extract does not produce a pressor substance when incubated with ox-serum substrate. But eel kidney extract produces an angiotensin-like substance under the same conditions (Chester Jones et al, 1966).

Chromatogram studies of angiotensin-like substances have shown that teleost angiotensins give two peaks on the chromatogram while mammalian angiotensin gives only one (Nakajima, Nakayama and Sokabe, 1971). Substances produced by the glomerular kidney and the

Corpuscles of Stannius have a common peak of an extremely basic site (op.cit., 1971). Teleost angiotensins are also more susceptible to proteases than are mammalian angiotensins (op.cit., 1971).

There also exists a difference between the Corpuscles of Stannius and the juxtaglomerular cells in their response to a hypercalcemic state. The Corpuscles of Stannius appear to be stimulated by hypercalcemia (Chester Jones et al, 1969) and show signs of hypertrophy when exposed to hypercalcemic conditions for long periods of time e.g. interrenalectomized eels in sea-water (op.cit., 1969). However, the J.G. cells appear to be inhibited by hypercalcemia and renin release decreases (Michelakis, 1971a). But the Corpuscles of Stannius and the J.G. cells show a similar response to hypocalcemia. The corpuscular cells show signs of atrophy when exposed to prolonged hypocalcemia while the rate of renin release from the J.G. cells decreases in response to hypocalcemia (Chester Jones et al, 1969; Michelakis, 1971a).

The J.G. cells are found in all three species of *Anguilla*, these being *Anguilla anguilla*, the European eel (Olivereau and Lemoine cited in Krishnamurthy and Bern, 1969); *Anguilla japonica*, the Japanese eel (Sokabe et al, 1969); and *Anguilla rostrata*, the North American eel (Capréol and Sutherland, 1968). Indeed, the Corpuscles of Stannius do not correspond with the criteria selected for identifying J.G. cells, these criteria being that they occur in the media of the afferent arterioles and occasionally in the efferent continuing with but replacing the smooth muscle layer, that their nuclei be rounded

rather than elongate and that their cytoplasm be well granulated, these granules staining with Bowie's stain (Capréol and Sutherland, 1968).

Despite the fact that saline extracts of the Corpuscles of Stannius appear to resemble renin, there are basic dissimilarities between corpuscular "renin" and kidney renin. The lack of reaction with ox-serum substrate by corpuscular tissue, the dissimilarity on chromatographic analysis, the dissimilarity of staining between the Corpuscles of Stannius and the J.G. cells and the stimulatory response of the corpuscles to hypercalcemia lead to the conclusion that the secretion(s) of the Corpuscles of Stannius are not primarily renin. It would appear that there is a renin-like substance in the corpuscles but it also appears that this "renin" is a much modified form. Also, even if part of the secretion(s) of the Corpuscles of Stannius were renin, any contribution made by the corpuscles to the renin-angiotensin system would be redundant and of little importance. The kidney of the eel is quite well supplied with renin and displays greater renin activity per unit weight than do the Corpuscles of Stannius (Chester Jones et al, 1969). Since the kidney is so much greater in mass than the Corpuscles of Stannius it would appear that the amount of renin contributed by the corpuscles would be negligible.

3. ROLE OF THE CORPUSCLES OF STANNIUS

While the precise role of the Corpuscles of Stannius is yet to be defined this work indicates that the apparent pressor

effect of the corpuscles may be attributed to their role in calcium homeostasis. This disagrees with the earlier findings of Chester Jones et al, (1966) who have shown the presence of a pressor substance in eel corpuscular extract and concluded this was a form of renin. While this is possible, much recent evidence indicates that the role of the corpuscles in the renin-angiotensin system is negligible. The dissimilarity in origin, the corpuscles arising from the mesonephric tubules and the J.G. cells arising from modified arterial smooth muscle cells, is another indication that the corpuscles are not part of the renin-angiotensin system. The lack of a hypotensive response to Stanniectomy when eels are adapted to an acalcemic environment is a strong indication that the "renin" of the corpuscles is not primarily a pressor agent, since a hypotensive response to Stanniectomy should result regardless of environment if the corpuscular secretions are a pressor substance. It is possible that the corpuscular substance is a pressor agent in extra-physiological doses in a manner analogous to vasopressin which is a pressor agent only in very high (extra-physiological) doses, but is primarily involved with water retention at the kidney tubular level.

The significant negative correlation between plasma calcium levels and blood pressure in Stanniectomized eels adapted to tap-water, twenty-four hour Stanniectomized eels infused with calcium and intact eels infused with calcium indicate that the decrease in blood pressure following Stanniectomy is a result of disturbed calcium homeostasis. This is emphasized by the hypotensive

response to high plasma calcium of intact eels infused with calcium.

From previous work and to a lesser extent this work, it would appear that the plasma ionic calcium fraction is the active fraction. Total plasma calcium is divided into three separate groups: (1) plasma ionic calcium, (2) diffusible bound-calcium and (3) non-diffusible protein-bound calcium. The result of the citrate infusion (Tables XVIII and XIX) suggest that it is the plasma ionic calcium which is the active fraction. Low plasma ionic calcium levels result in a decrease in blood pressure and very low plasma ionic calcium levels are associated with a significant decrease in blood pressure. Since a significant decrease in blood pressure may be obtained by binding the ionic calcium fraction without significantly altering the total plasma calcium and since citrate infusion should not affect the protein-bound calcium or the diffusible bound calcium it appears that the ionic calcium fraction is the active fraction. A highly significant ($P < 0.01$) negative correlation which was established between plasma ionic calcium and blood pressure also tends to indicate that the ionic calcium is the active fraction.

The role of the Corpuscles of Stannius in the regulation of plasma ionic calcium is unclear. This work seems to indicate that the corpuscular extract contains some fraction which binds calcium or causes plasma ionic calcium binding since injection of corpuscular extract results in a significant decrease in plasma ionic calcium. Also, Stanniectomized eels show an increase in plasma ionic calcium levels above that of sham-operated animals although this increase

was not significant in the Stanniectomized eels adapted to an acalcemic environment. Chan and Chester Jones (1968) have found that Stanniectomy of European eels results in a significant increase in the ultra-filtrable fraction of plasma calcium after one week post-operative time but plasma ionic calcium was not measured. However, the total proportion of ultra-filtrable to total plasma calcium showed a significant decrease in Stanniectomized European eels. This suggests that the calcium-binding capacity of the plasma proteins is quite large. It has also been found that the Corpuscles of Stannius tend to inhibit calcium uptake from the ambient environment (Fontaine et al, 1972).

If the corpuscular secretions do bind plasma ionic calcium, then this could be an explanation for the decrease in systemic blood pressure observed in Stanniectomized eels after three days post-operative time (Chester Jones et al, 1966) and for which the mechanism proposed as a result of this work can offer no explanation. The lack of corpuscular secretion(s) binding activity would result in a greater proportional increase in plasma ionic calcium since the binding capacity of the plasma proteins is not unlimited. Therefore, since plasma ionic calcium appears to be the active fraction, an increase in this fraction would result in a decrease in blood pressure. However, the evidence for a role of the Corpuscles of Stannius in the binding of plasma ionic calcium is controversial and this hypothesis is thus largely speculative.

To recapitulate, this work indicates that the Corpuscles

of Stannius do not possess a pressor substance of any great physiological importance. The hypotensive response to Stanniectomy appears to be solely a result of disturbed calcium homeostasis. These findings do not totally agree with previously published work on the role of the Corpuscles of Stannius in blood pressure, but other investigations have been done on the possibility that the corpuscles possess a pressor substance and are a part of the renin-angiotensin system. This work tends to agree with these later findings which indicate that the Corpuscles of Stannius are not part of the renin-angiotensin system and that if they do contain a form of renin it is a much modified form. While this work clarifies the role of the Corpuscles of Stannius to some extent, the mechanism by which hypercalcemia results in a decrease in blood pressure and the precise role of the corpuscles in calcium metabolism remain obscure. This requires that further work be done on this problem.

SUMMARY AND CONCLUSIONS

1. Stanniectomy of fresh-water adapted eels results in a decrease in blood pressure and an increase in plasma calcium levels. These effects are not seen when Stanniectomized eels are placed in an acalcemic environment.
2. Infusion of calcium into intact eels results in an increase in total plasma calcium and a decrease in blood pressure when total plasma calcium levels are approximately those of two-week Stanniectomized eels.
3. Hypocalcemia caused by infusion of sodium citrate results in a decrease in blood pressure. However this decrease is only seen when plasma ionic calcium levels are below 1.0 meq/l.
4. There are several possible mechanisms for the action of calcium which would result in a decrease in blood pressure; an inhibition of cortisol biosynthesis, a direct inhibition of the cardiovascular system, or an inhibition in the rate of renin release from the kidney. It would appear that the latter two mechanisms are of greater importance than the first action.
5. The Corpuscles of Stannius do not appear to be homologous with juxtaglomerular cells and appear to give no pressor response in eels when a saline extract of the corpuscles is injected intravenously in doses of one corpuscle per kilogram body weight.

6. The decrease in blood pressure brought about by surgical removal of the Corpuscles of Stannius appears to be a consequence of disturbed calcium homeostasis rather than the loss of a pressor substance. At this time the exact mechanism of action of calcium on blood pressure is unknown and more work is required to elucidate the mechanism of action.

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APPENDIX I

THE EFFECT OF STANNIECTOMY AND ENVIRONMENT ON PLASMA

SODIUM LEVELS IN AMERICAN EELS ^a

GROUP	PLASMA SODIUM LEVELS mEq/L
Stanniectomized (Tap-water Adapted)	114.22 ± 8.3 (9)
Sham-Operated (Tap-water Adapted)	126.44 ± 21.72 (9)
Intact Controls (Tap-water Adapted)	146.44 ± 17.51 (9)
Stanniectomized (Acalcemic-water Adapted)	118.83 ± 5.89 (12)
Sham-Operated (Acalcemic water Adapted)	155.78 ± 7.21 (9)
Intact Controls (Acalcemic water adapted)	144.89 ± 12.20 (9)

^a values are means ± S.E.M. (N)

APPENDIX II

PLASMA SODIUM LEVELS BEFORE AND AFTER INFUSION OF VARIOUS TEST SOLUTIONS AND IN CONTROL GROUPS ^a

GROUP	PLASMA SODIUM (mEq/l)	
	BEFORE INFUSION	AFTER INFUSION
b		
Tap-water Controls A	167.11 ± 5.4 (9)	147.11 ± 11.05 (9)
B	147.11 ± 11.05(9)	152.89 ± 5.49 (9)
Calcium Chloride Infusion (Second Experiment)	162.89 ± 22.07(9)	145.5 ± 15.0 (9)
b		
Distilled water Controls A	242.6 ± 24.19(10)	170.56 ± 19.57 (9)
B	170.56 ± 19.57(9)	172.0 ± 22.0 (9)
Hypo- and Hyper-Calcemia Experiment: Citrate Infusion	162.25 ± 7.67(8)	163.38 ± 21.9 (8)
Calcium Chloride Infusion	163.38 ± 21.9 (8)	168.25 ± 7.03 (8)
Angiotensin II Injection	156.67 ± 21.68(6)	158.40 ± 27.8 (5)
Corpuscular Saline Extract	158.4 ± 27.8 (5)	170.0 ± 34.29 (6)

a values are means ± S.D.M. (N)

b For control groups: before Infusion = at the beginning of the of the recording period.
after Infusion = at the end of the recording period.

A and B refer to Period A and Period B respectively.