STEROIDS
ARTERIAL PRESSURE
AND
THE BARORECEPTORS

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This thesis has not been submitted for a degree to any other university.

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SUMMARY

Intravenous infusions of aldosterone (6 µg/kg/hr) raised the arterial pressure of decerebrate rabbits 20% in five hours. Similar infusions of aldosterone into progesterone pre-treated animals result in a 45% rise in arterial pressure, into salt fed animals, 30% rise, and into salt deprived animals, 10% rise. The arterial pressure of progesterone pre-treated animals rose 15% during a saline infusion of five hours duration.

Surgical interference with the ability of both carotid baroreceptors to detect elevated pressure resulted in a 38% chronic hypertension in rabbits whose arterial pressure was measured daily for fifty days. The ability of noradrenaline infusions (1-2 µg/kg/hr) to maintain elevated arterial pressure in decerebrate rabbits was greatly enhanced by nembutal anaesthesia. At the same time the sensitivity of the baroreceptor reflex declined considerably, after the nembutal administration. The exposure of the carotid sinus to aldosterone caused the arterial pressure to rise even though the remainder of the circulation was protected from the steroid. When the carotid sinus was protected from aldosterone, which was allowed to circulate throughout the rest of the body, the arterial pressure failed to rise. Aldosterone infusions were ineffective in raising the arterial pressure in nembutal anaesthetised animals.

Infusion of cholesterol emulsions (0.3 mg/kg/hr) caused the arterial pressure of decerebrate rabbits to rise 45% in four hours. Infusion of a similar quantity of cholesterol in plasma dialysate resulted in a rise in arterial pressure of 15% in four hours. An inert emulsion of talc had no effect on arterial pressure when infused. The feeding of a diet high in unsaturated fats to rabbits raises the arterial pressure with no elevation of blood cholesterol concentration.

It is concluded that aldosterone may contribute to hypertension in primary aldosteronism, malignant hypertension, some

cases of renal and essential hypertension, and the hypertension of pre-eclampsia. It is concluded that aldosterone produces its hypertension by acting upon the baroreceptors. It is concluded that cholesterol may be important in the generation of hypertension. It is suggested that the cholesterol concentration in the blood which is important is the small quantity of chemically free cholesterol rather than the protein bound or esterified moiety.

Acknowledgements

These are given in chronological order.

The Canadian University Services Overseas are my sponsors here and have provided me with a relatively trouble free existence.

Prof. David Horrobin arrived here and cheerfully asked me if I would like to cannulate some arteries and measure some blood pressures. The resulting partnership has been surprisingly productive. I have never before had anyone pay attention to my mad ideas with so much tolerance and interest. Mainly, I thank David for transforming a few wild inspirations into workable experimental designs, and for steadfast encouragement where others would probably have faltered.

Gregory Bizoza managed against unfavourable odds to keep the technical side of the department from tumbling into chaos.

Ieuan Lloyd arrived in confusion and left the same way. In between some hard work was done. I thank him for his help, and am proud to share authorship of a number of papers with him.

Kathleen Muiruri inherited Gregory's domain, and has certainly managed to maintain the high standard set by him. I am indebted to Kathleen's technical assistance and expertise.

Ned Durkin arrived when my soul was dispirited. I am grateful for the resulting inflation of my spirits, for the friendship which ensued, and, not least, for the occasional use of his talented kidneys.

I cannot properly express my gratitude for the existence of a fine library at Makerere University, which, though it was located over 400 miles from Nairobi, was the only place where I could read the larger number of references quoted in this thesis without the frustration of not being able to locate some.

I have made extensive use of the following review books:

"Reflexogenic Areas of the Cardiovascular System" C. Heymans & E. Neil, 1958, Churchill.

"Arterial Disease" J.R.A. Mitchell & C.J. Schwartz, 1965, Blackwell.

"Renal Hypertension" I.H. Page & J.W. McCubbin, 1965, Year Book Medical Publishers.

"Neurogenic Hypertension" C.J. Dickinson, 1965, Blackwell.

"Hormones and Hypertension" W.M. Manger, 1966, Thomas.

"Baroreceptors and Hypertension" P. Kezdi, 1967, Pergamon.

"High Blood Pressure" G. Pickering, 1968, Churchill.

"Renin and Hypertension" M.R. Lee, 1969, Lloyd-Luke.

"Scientific Foundations of Obstetrics and Gynecology" E.E. Philipp, J. Barnes & M. Newton, 1970, Heinemann.

"Experimental Cardiovascular Diseases" H. Selye, 1970, Springer-Verlag.

Very special thanks go to Elaine Parthenais who somehow managed to organise, proofread and type my manuscript. The patience she exercised with me was barely short of angelic.

Explanatory note

Most of the experimental work reported in this thesis has already appeared in the form of articles in various journals. The presentation of a thesis describing work which has been largely published previously poses something of a philosophical problem. Usually, when writing a thesis, one refers to published papers as if they were established facts. Since most of the results presented in this thesis have been published, they are as much established fact as any other papers to which one might refer. However, a thesis is supposed to be a presentation of an idea which is not yet proven. For this reason, the papers forming the bulk of the experimental results contained in this thesis will not be directly referred to in the text.

The published papers upon which most of this thesis is based are listed below. Reprints of these articles are bound in with the thesis.

Lancet, May 9, 973, 1970.

J. Obs. Gyne. Br. Commw., 77, 928, 1970.

J. Endo. 50, 653, 1971.

Am. J. Obs. Gyne. 110, 994, 1971.

Cardiov. Res. 6, , 1972.

Br. J. Exp. Path. 1972 (in press)

CHAPTER I: INTRODUCTION

Arterial hypertension is defined as a blood pressure which exceeds what is considered normal for a person of a given age and sex. Hypertension is generally divided into two broad categories: elevated blood pressure whose causes are more or less understood, and "essential" hypertension, for which there is no known cause.

Certain disease processes which are caused by the hypersecretion of a steroid hormone such as Cushing's syndrome and Conn's syndrome are also characterised by elevated blood pressure. Overdosage of desoxycorticosterone (DOC) (Knowlton, Loeb, Stoerk, and Seegal, 1947; Selye, Stone, Timiras, and Schaffenburg, 1949) is well known for the resulting blood pressure elevation, and there is a growing body of evidence that oral contraceptives containing steroid agents can also result in hypertension in certain individuals (Woods, 1967; Weir, Briggs, Mack, Taylor, Browning, Naismith, and Wilson, 1971). Finally, there is a long standing belief that the incidence of cardiovascular disease is decreased in individuals who reduce the cholesterol content of their diet (Leren, 1966, 1968).

This thesis concerns itself primarily with the steroids aldosterone, progesterone, and cholesterol, and their effects upon blood pressure. The results suggest causes for the raised blood pressure seen in Conn's syndrome, in toxaemia of pregnancy, and perhaps for some forms of renal hypertension. In addition, some new ideas are presented upon the relationship between elevated blood pressure and arterial disease.

Primary aldosteronism was defined by Conn (1961) as a syndrome of mineralocorticoid excess, induced by adrenal cortical elaboration of excessive quantities of aldosterone, and characterised by abnormally large urinary excretion of aldosterone, with normal excretion of urinary 17-hydroxycorticoids and 17-ketosteroids. The basic findings in the disease are severe potassium loss, and hypertension, most other features being secondary to these two. Aside from these signs, diagnosis

of primary aldosteronism depends upon the patient demonstrating a low plasma renin activity despite a low sodium intake.

Two controversies rage about primary aldosteronism. The first concerns the contribution of primary aldosteronism to the overall picture of hypertension. Conn in several publications claims that about 20% of all "essential" hypertensives are actually suffering from aldosteronism (Conn, Rovner and Cohen, 1965; Conn, Rovner, Cohen and Nesbit , 1966), and hence treatable by surgery. In contrast, Laragh and his group maintain that the disease is very rare indeed, seldom appearing in their clinics (Laragh, Sealey, and Sommers, 1966; Ledingham, Bull, and Laragh, 1967).

The other problem centers around the agent responsible for the hypertension. In general, the obvious culprit, aldosterone, has been discounted because of its apparently weak pressor activity (evidence for this will be reviewed below). Since renin and angiotensin are present in normal or lower than normal amounts, they cannot be responsible. However, in the majority of cases of Conn's syndrome, surgical removal of the aldosterone secreting tumour restores the blood pressure and the plasma potassium to normal. Thus one is left with little choice but either to show that aldosterone is responsible for the hypertension, or to positively disprove this and suggest another agent.

A portion of this thesis will be devoted to showing that aldosterone may indeed be responsible for the hypertension seen in Conn's syndrome. In addition, a hypothesis will be offered to explain the often seen delay in the normalisation of the arterial pressure following surgical removal of the aldosterone producing tumour.

By standard medical textbook definition, pre-eclampsia is characterised by hypertension, proteinuria, and oedema. In actual practice, obstetricians being cautious men, any one, or at most two of these symptoms may be sufficient to produce a diagnosis of pre-eclampsia, and probably some form of treatment.

Investigation of the cardiovascular system in pre-eclampsia has provided undramatic results. Cardiac output is normal. Blood volume is reduced. Cerebral, hepatic, and cardiac blood flows are also normal. Renal and uterine blood flows may be reduced, while blood flow to the limbs may be somewhat increased (Pickering, 1968, p. 604; MacGillivray, 1969). Even though the original investigations which provided this picture were done a relatively long time ago, with at times questionable techniques, clearly there are no outstanding circulatory disorders in pre-eclampsia apart from the hypertension. This hypertension must be the result of increased peripheral resistance (Pickering, 1968).

The major endocrine abnormality which is noted in pre-eclampsia is increased urinary excretion of chorionic gonadotrophin, which may be almost doubled. The serum concentration of the hormone is also elevated, but there seems to be no correlation between the severity of the symptoms and the hormonal level. Because of this, Pickering (1968) argues against the involvement of this hormone in pre-eclampsia.

In a recent prospective study, early in pregnancy plasma renin was found to be elevated in women who later developed pre-eclampsia (Gordon, Parsons and Symonds, 1969). However, renin, angiotensin, and aldosterone levels are all normal or reduced in established pre-eclampsia (Rinsler and Rigby, 1957; Sims, Meeker, Gray, Watanabe, and Solomon, 1964; Brown, Davies, Doak, Lever, Robertson, and Trust, 1966).

Urinary pregnanediol excretion is reduced in pre-eclampsia and this has been taken to mean that the plasma concentration of its precursor, progesterone, is also reduced. However, the rate of conversion of progesterone to pregnanediol is also reduced in pre-eclampsia (Macnaughton and Greig, 1965), which could mean that the plasma concentration of progesterone may actually be increased (Horrobin, 1968, 1971).

The standard treatment regime for pre-eclampsia includes weight control, salt restriction, sedatives, hypotensive agents

and diuretics, though the latter are falling out of favour nowadays. Oddly enough, despite the near universality of these treatments, there are remarkably few convincing theories as to what causes the disease. Even more unusual is the observation that these standard treatments have no effect upon the survival of the infant born of a pre-eclamptic mother, and relatively little effect upon the survival of the woman either (Brewer, 1962, 1969-1971 personal communications; Pickering, 1968; MacGillivray, 1969; Horrobin, 1971). These treatments do tend to reduce both the elevated blood pressure, and the oedema, but it appears that by the time the disease is recognised, and treatment started, the damage has already been done (Horrobin, 1971).

Robinson (1958) found that women who were instructed to increase their intake of dietary salt had 1/3 the incidence of pre-eclampsia of another group who were instructed to take a low salt diet. On first glance, the administration of sodium to pregnant women is heretical, and as might be expected, this unusual and effective prophylaxis has gone quite unheeded.

Part of this thesis will be devoted to advancing the hypothesis that progesterone may be at least partly responsible for the hypertension of pre-eclampsia. Some possible reasons for the effectiveness of Robinson's prevention of pre-eclampsia will also be proposed.

The relationship between arterial health, arterial pressure and the concentration of cholesterol in the circulation at first glance appears to be fairly simple. It has been shown in many surveys of populations that either separately or combined, elevated blood cholesterol and elevated arterial pressure can result in a greatly increased incidence of death through arterial disease, and particularly coronary thrombosis (Leren, 1966, 1968, 1970; Dawber, Kannel, Revotskie, and Kagan, 1962; Karvonen, 1962). The two factors are additive in their effect upon arterial disease. There also exists a relatively sharp division between blood pressures which tend to increase the

incidence of arterial disease, and those which seem to have no effect. Similarly, blood cholesterol concentrations above 260 mg % increase the likelihood of arterial disease greatly, while those slightly below this figure seem to have little or no effect.

It is not proposed to review the literature pertaining to the general field of arterial disease because it is vast, requires specialised medical knowledge to be properly understood, and because there is a tendency to loose terminology with respect to words like atherosclerosis, arteriosclerosis, atheroma, etc.

It is hoped to produce satisfactory indirect evidence that arterial disease of a particular type may be itself responsible directly for causing an increased arterial pressure. It will be shown that under certain circumstances cholesterol may be potently hypertensive. It will be suggested that absolute blood concentrations of cholesterol may in themselves be meaningless when used as indicators of the probability of arterial disease, and that other factors such as the binding ability of the blood for cholesterol may be far more important.

CHAPTER II: ALDOSTERONE

Introduction

Prior to the 1950s desoxycorticosterone (DOC) was the only mineralocorticoid known, although the existence of other steroids with sodium retaining properties was suspected (Conn, 1949). DOC was also well known for its hypertensive action (Knowlton, Loeb, Stoerk, and Seegal, 1947; Selye, Stone, Timiras, and Schaffenburg, 1949). Aldosterone was discovered in 1952, and its mineralocorticoid potency found to be very much greater than that of DOC (Simpson, Tait, and Bush, 1952; Tait, Simpson, and Grundy, 1952). However, studies of its effects upon arterial pressure were at first somewhat inconclusive.

Kumar, Hall, Nakashima, and Gornall (1955) showed that the daily administration of 0.25 µg of aldosterone caused a significant rise in the arterial pressures of rats after two months. However, Gross, Loustalot, and Meier (1955) were unable to show any rise in arterial pressure in rabbits given 40 µg aldosterone daily although this was a considerably larger dose on a weight basis. From the vantage point offered by the 1970s, one is tempted to suspect that both of these groups were worried about the discrepancy between their results, because in 1957, both groups published again, slightly modifying their first results. Thus, Kumar et al. (1957) then claimed that aldosterone was only very slightly hypertensive, while Gross et al. (1957) decided that it actually did increase arterial pressure. Nevertheless, Gaunt, Ulsamer, and Chart (1957) were unable to detect any rise in arterial pressure in rats after seven months treatment with 0.5 µg daily. In 1960, Gornall, Grundy, and Koladich claimed a 20% rise in arterial pressure in rats given 0.5 µg daily for three to six months. Masson, Mikasa, and Yasuda (1962) gave rats the gigantic dose of 0.2 mg daily, and this produced a 30% increase in arterial pressure, and a 50% rise if saline was substituted for drinking water. Since the difference between the salt fed rats and those on a normal diet was not statistically significant, they

concluded that aldosterone hypertension was largely independent of salt loading. Similar results were reported by Hall and Hall (1965).

The conclusion of a decade of investigation on the effects of aldosterone on arterial pressure was that aldosterone is not a potent blood pressure increasing agent, that it causes arterial pressure to rise slowly over a period of several weeks, and that salt loading has relatively little effect upon this rise. It was also made clear that if both aldosterone and DOC were administered in doses of equal sodium retaining potency, the latter is by far the more potent hypertensive agent (Gross and Schmidt, 1958).

In 1955, J.W. Conn described the syndrome which now bears his name. He attributed the main symptoms, hypokalemia, hypernatremia, and hypertension to the excessive secretion of aldosterone (Conn, 1961). Furthermore, he suggested that some 20% of patients classed as "essential hypertensives" may actually be suffering from primary aldosteronism, and hence surgically curable (Conn et al., 1965; Conn et al., 1966). Laragh and his group (Laragh, Sealey, and Sommers, 1966; Ledingham, Bull and Laragh, 1967) disagree with Conn. They claimed that primary aldosteronism is a rare disease which they diagnosed very seldom in their clinics.

Elevated aldosterone secretion is also seen in "essential" hypertension (Garst, Shumway, Schwartz, and Farrell, 1960; Genest, Nowaczynski, Koiw, Sandor, and Biron, 1960). These authors made no claim that aldosterone was responsible for the elevated arterial pressure.

Genest and his colleagues (Genest, Koiw, Nowaczynski, and Sandor, 1960; Genest, Nowaczynski, Koiw, Sandor, and Biron, 1960; Genest, 1961) and Laragh and his colleagues (Laragh, Angers, Kelly, and Lieberman, 1960; Ames, Borkowski, Sicinski, and Laragh, 1965) showed that infusions of angiotensin were potent stimulators of aldosterone production. Genest (1961) also observed that aldosterone levels were raised in benign,

malignant, and renal hypertension. It is now well known that aldosterone secretion and excretion rates are elevated in malignant hypertension (Laragh, Ulick, Januszewicz, Deming, Kelly, and Lieberman, 1960; Laragh, Sealey, and Sommers, 1966; Brown, Davies, Lever, and Robertson, 1966). This is an example of secondary aldosteronism since it is the elevated renin and angiotensin levels in the plasma which are probably responsible for the overproduction of aldosterone. In fact, the aldosterone production in malignant hypertension is about 50% or more greater than that seen in primary aldosteronism (Conn, 1961).

Animal experiments have cast considerable doubt as to the ability of aldosterone to raise arterial pressure. For this reason, certain investigators have preferred to blame other agents to explain the hypertension of Conn's syndrome. Crane and Harris (1966) have suggested that DOC may be the cause, and Pickering (1968, p. 581) has proposed that another, as yet unidentified, adrenal hormone secreted in parallel with aldosterone may be responsible. However, in view of the demonstrated elevation of aldosterone in Conn's syndrome, and the remission of symptoms which usually follows surgical removal of the tumour, it is hard to dismiss the importance of aldosterone in this, and other diseases discussed above. Experiments are reported here which demonstrate a rapid hypertensive action of intravenously infused aldosterone. The amount of the infusion was made to be of approximately the same order of magnitude as the maximum demonstrated aldosterone secretion rates seen in Conn's syndrome and malignant hypertension.

Methods

Rabbits weighing between 1.5 and 3.0 kg of either sex were used. They were kept in a run before the experiments, fed lucerne and commercial rabbit pellets, with water available ad libitum. Male and female rabbits were kept separately to avoid the inadvertent use of pregnant animals.

The experimental animal was anaesthetised with ether, and then a tracheal cannula was inserted. Mid-collicular

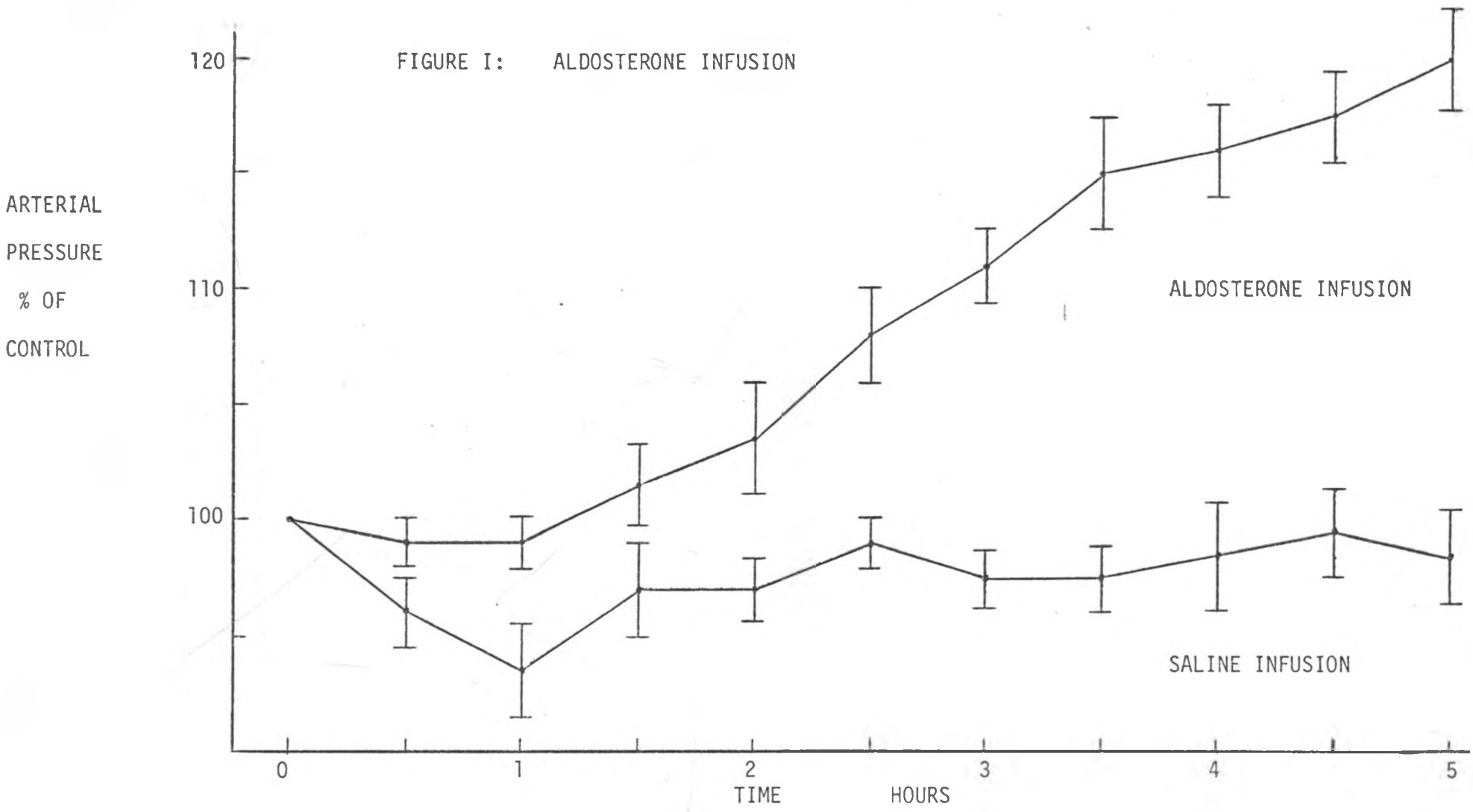
TABLE I: Arterial blood pressures at ½ hourly intervals in untreated decerebrate rabbits following intravenous saline infusion.

TIME	BLOOD PRESSURES								MEAN % OF STARTING PRESSURE		
									%	S.D.	S.E.
0	76	89	80	88	80	92	83	70	100		
½	70	88	74	88	74	92	83	64	96.00	4.07	1.44
1	68	90	66	88	74	86	81	62	93.50	6.11	2.16
1½	75	89	68	86	75	96	83	68	97.12	5.66	2.00
2	75	91	72	84	78	92	79	67	97.00	3.66	1.29
2½	75	90	76	88	84	92	80	68	99.12	3.18	1.12
3	70	85	76	86	78	93	82	72	97.75	3.45	1.22
3½	75	92	75	84	76	86	79	72	97.37	3.81	1.35
4	77	95	80	76	78	92	77	73	98.62	6.54	2.31
4½	78	89	79	84	78	93	76	77	99.75	5.20	1.84
5	72	91	81	82	77	93	76	77	98.75	5.94	2.10
	Mean starting pressure (mm Hg)								82.25	7.30	2.58

TABLE II: Arterial blood pressures at ½ hourly intervals in untreated decerebrate rabbits following intravenous aldosterone infusion.

TIME	BLOOD PRESSURES										MEAN % OF STARTING PRESSURE		
											%	S.D.	S.E.
0	88	80	95	79	85	88	72	67	82	80	100		
½	86	83	96	77	86	90	67	65	81	77	98.90	3.21	1.01
1	84	84	95	77	89	88	71	64	78	76	98.80	3.82	1.20
1½	92	90	96	78	91	84	72	63	84	79	101.70	5.55	1.75
2	90	96	94	78	94	95	69	64	84	80	103.40	7.57	2.39
2½	91	98	95	82	95	100	76	68	92	84	108.10	7.04	2.22
3	94	98	98	86	98	98	78	74	93	89	111.20	5.34	1.68
3½	104	96	95	87	100	100	79	78	106	90	114.80	7.59	2.40
4	104	96	100	88	99	100	79	78	104	94	116.30	6.51	2.06
4½	110	100	105	88	99	101	82	81			117.50	5.55	1.96
5	114	104	110	89	100	103	84	80			120.12	6.40	2.26
	Mean starting pressure (mm Hg)										81.60	8.09	2.56
	Mean starting pressure (mm Hg) all normals										81.88	7.53	1.77

FIGURE I: ALDOSTERONE INFUSION



decerebration was then carried out, and the ether removed. D-tubocurarine was administered in an amount sufficient to prevent reflex movements, and thereafter in topping up doses according to need. The femoral vein and artery were both cannulated with nylon siliconised ("Siliclad" applied) cannulae. The arterial pressure was continuously monitored with a Devices M4 pen recorder from strain gauge pressure transducers. Infusions were made with a B. Braun (Melsungen) syringe infusion pump set to deliver 2.0 ml/minute.

In these experiments, arterial pressure was allowed to stabilise for one hour prior to the start of the infusion. The infusion consisted of isotonic saline for the controls, or aldosterone (Aldocorten, Ciba) in isotonic saline for the experimentals, both at the rate of 2.0 ml/min. The aldosterone concentration of the infusion was adjusted according to the weight of the animal so that it would deliver 6 µg/kg/hr. This rate of infusion is approximately equivalent to the highest rates of secretion of aldosterone seen in humans (10,000 µg/24 hr), in malignant hypertension (Laragh, Sealey, and Sommers, 1966), in pregnant women on a restricted sodium intake (Watanabe, Meeker, Gray, Sims, and Solomon, 1963), and in liver failure (Laragh, Cannon, and Ames, 1964). At the beginning of the infusion, one ml was given rapidly as a priming dose.

Results

TABLES I, II

FIGURE I

In the control group given saline infusions, the arterial pressure remained steady at roughly 98% of its starting value for the duration of the five hour infusion.

In the aldosterone infused animals, the arterial pressure remained steady for 1-1 1/2 hours, and then began a steady rise, increasing after five hours to 20% above its starting value.

The mean arterial pressure at the start of the infusion in the two groups of animals was 82.25 $\pm$ 2.6 (means are followed

by their standard error throughout the thesis) in the controls, and 81.60 ± 2.6 in the aldosterone infused.

CHAPTER III: ALDOSTERONE AND PROGESTERONE

Introduction

Only relatively recently has the importance of progesterone in human hypertension been suspected. Woods (1967) noticed that hypertension occurred in six of his patients following oral contraceptive therapy. Their blood pressures returned to normal after the medication was withdrawn. Similar observations were made by Laragh, Sealey, Ledingham, and Newton (1967), and have been confirmed by others. It is now clear that hypertension only occurs in a minority of women using oral contraceptives. Weir, Briggs, Browning, Mack, Naismith, Taylor, and Wilson (1971) conducted a prospective study in which a number of oral contraceptive preparations were studied with respect to their effects upon arterial pressure. They found that out of 66 women in their trial, 50 showed a rise in arterial pressure after one year although this averaged only about 5%.

Lim, Lumbers, Walters, and Whelan (1970) found that intravenous infusions of oestrogens raise arterial pressure, and therefore claimed that it was the oestrogenic component of the oral contraceptive which is responsible for the elevation of arterial pressure. This contrasts with the results of Ueland and Parer (1966) who showed that, although intravenous oestrogen infusions cause large increased in cardiac output of ewes, at the same time, there was a slight drop in arterial pressure. Also Weir et al. (1971) disagree with Lim et al. (1970) on the grounds that there was no difference between the arterial pressure changes in women on oral contraceptive preparations containing various quantities of oestrogens.

Horrobin (1968), and Horrobin and Lloyd (1970), showed that chronic treatment of rabbits with progesterone, in amounts similar to those encountered in human pregnancy at term resulted in an increase in blood pressure. This finding has been confirmed by Weir et al. (1971). The mechanism of this increase was puzzling, because lower doses of progesterone result in

hypotension (Armstrong, 1959; Horrobin, 1968). Nevertheless, it is fairly clear that progesterone, if present in sufficient concentration, can exert a hypertensive action, and such concentrations are present in human pregnancy at term (Horrobin, 1971). Equivalent concentrations of progestins are apparently supplied in oral contraceptive therapy (Weir et al., 1971).

Laragh, Sealey, Ledingham, and Newton (1967) confirmed the observation that oral contraceptives can produce hypertension. Furthermore, they showed that oral contraceptive therapy increased renin, angiotensin, and aldosterone levels in both hypertensive and normotensive women. Walters and Lim (1970), and Catt, Cran, Zimmet, Best, Cain, and Coghlan (1971) confirmed the increase in renin and angiotensin following oral contraceptive therapy.

Progesterone antagonises the sodium retaining property of aldosterone (Martin and Mills, 1956; Landau and Lugibihl, 1958). This saluretic action may well explain the increased aldosterone secretion, and increased secretion of renin and angiotensin noted above. The actual levels of aldosterone which are seen in pregnancy average about 4-5 times normal, and if the sodium intake is restricted during pregnancy, they may rise to 50 times normal (Watanabe, Meeker, Gray, Sims, and Solomon, 1963).

Since progesterone levels in pre-eclampsia may be elevated above the normal (Horrobin, 1971), and aldosterone levels are already high in pregnancy as a result of progesterone induced saluresis, it was of interest to investigate any possible synergism between these two steroids upon the arterial pressure.

Methods

All of the animals in this group were kept caged for two weeks prior to the acute experiment, and injected intramuscularly with 25 mg of progesterone in oil (Antigen Progesterone, BDH Progestin, or Organon Progesteronum) every morning (18 animals) or 2.5 mg progesterone (5 animals). The higher dose is approximately equivalent to the rate of secretion of progesterone

TABLE III: Arterial blood pressures at ½ hourly intervals in progesterone (25 mg/day) pretreated decerebrate rabbits following intravenous aldosterone infusion.

TIME	BLOOD PRESSURES										MEAN % OF STARTING PRESSURE		
											%	S.D.	S.E.
0	80	62	71	92	78	80	91	60	67	88	100		
½	80	58	72	95	80	82	92	62	70	89	101.40	2.98	0.94
1	84	76	73	104	83	83	91	63	64	86	105.30	7.57	2.39
1½	84	77	77	104	90	90	95	67	82	98	111.80	6.74	2.13
2	88	84	88	106	108	92	96	72	77	97	118.70	10.64	3.36
2½	89	96	85	109	109	100	102	75	80	97	123.60	14.07	4.45
3	92	94	84	113	118	104	101	82	88	100	127.30	16.19	5.12
3½	94	92	90	112	117	111	103	90	93	105	132.40	14.20	4.49
4	104	94	93	114	120	117	104	94	97	116	138.40	14.17	4.48
4½	130	90	90	121	119	121	105	97	103	118	143.70	15.74	4.98
5	130	92	95	119	121	121	109	98	104	123	146.00	14.52	4.59
	Mean starting pressure (mm Hg)										76.90	11.59	3.66

TABLE IV: Arterial blood pressures at ½ hourly intervals in progesterone (25 mg/day) pretreated decerebrate rabbits following intravenous saline infusion.

TIME	BLOOD PRESSURES								MEAN % OF STARTING PRESSURE		
									%	S.D.	S.E.
0	87	80	73	68	103	65	65	60	100		
½	88	82	72	68	103	65	65	63	100.87	1.88	0.66
1	90	82	64	67	108	61	63	69	100.50	8.07	2.86
1½	100	83	75	71	114	62	62	68	103.87	6.68	2.36
2	104	82	71	71	117	64	69	69	107.50	7.98	2.82
2½	100	78	79	71	112	64	66	69	105.00	5.88	2.01
3	100	80	81	80	114	65	70	69	108.50	6.56	2.32
3½	100	80	80	83	125	70	79	69	112.87	8.42	2.98
4	100	84	82	83	123	73	83	71	115.25	8.17	2.89
4½	100	80	89	85	105	75		73	113.00	10.51	3.98
5	96	80	96	84	106	78		76	116.57	12.35	4.67
	Mean starting pressure (mm Hg)								75.12	14.29	5.06
	Mean starting pressure (mm Hg) all progesterone (25 mg/day)								76.11	12.49	2.94

TABLE V: Arterial pressures at ½ hourly intervals in progesterone (2.5 mg/day) pretreated decerebrate rabbits following intravenous aldosterone infusion.

TIME	BLOOD PRESSURES				
0	80	80	84	90	68
½	82	77	84	88	75
1	77	74	85	102	80
1½	72	71	87	120	83
2	76	76	88	128	94
2½	87	80	88	128	95
3	88	85	96	142	103
3½	92	88	93	157	102
4	91	86	93	156	104
4½	91	89	90	154	101
5	92	87	90	155	102

Mean starting pressure (mm Hg) 80.40

S.D. 8.04

S.E. 3.60

in women in the third trimester of pregnancy, 200-500 mg/24 hr (Hytten and Leitch, 1964). These animals were all given 1% saline to drink in order to prevent any rise in the arterial pressure due to the progesterone (Horrobin and Lloyd, 1970).

The animals were prepared for the acute experiments in the same way as the previous groups (Methods, CHAPTER II). They were infused with either 6 µg/kg/hr aldosterone in 2 ml/hr saline, or saline alone, 2.0 ml/hr.

Results

TABLES III, IV, V
FIGURE II

The ten animals which were treated with 25 mg/day of progesterone, and then infused with aldosterone showed a far greater rise in arterial pressure over the five hour infusion than did either the untreated, aldosterone infused animals described before, or the sodium loaded, aldosterone infused animals described in the next chapter. The arterial pressure began to rise at least 1/2 hour earlier and reached a level of 45% above its starting value in the five hours.

The five animals receiving only 2.5 mg/day of progesterone showed a far smaller average rise in arterial pressure, and it can be noted that whereas three animals showed substantial increases, two others showed very small increases.

The group of eight animals pretreated with 25 mg/day of progesterone and infused with saline serve as controls for this set of experiments. They showed a rise in arterial pressure of 15% above the starting level which was totally unexpected.

The mean starting blood pressure of the 18 progesterone treated animals was 76.11 ± 2.9, which compares to the starting pressure of the untreated animals of 81.88 ± 1.8, and these two are not significantly different ~~at the 90% level~~. ($p > 0.1$).

CHAPTER IV: ALDOSTERONE AND SODIUM

Introduction

Diets low in sodium content were first used to treat hypertensives in 1948. Kempner (1948) introduced the "rice diet" which also featured a low protein content. Schroeder (1948) claimed that any diet low in sodium would be just as effective in lowering the arterial pressure. Today, the treatment of hypertensives with diets which are either deficient in sodium, or normal diets coupled with diuretic therapy is common practice.

In otherwise normal people, the quantity of sodium ingested may affect the arterial pressure. Dahl and Love (1954) reported that individuals who tended to use a large amount of salt in their diet had a greater probability of being hypertensive than those who tended to use little salt. On the other hand, Miall (1959) found that in men there was no correlation between salt intake and blood pressure; but women with a high salt intake tended to have lower arterial pressures than those with a low salt consumption. Dahl (1961) studied the salt content of the diets of various populations, and their arterial pressures. He reported that there was a straight line relationship between the average salt intake of the population and the percentage of hypertensives in it. Thus, from these human studies, it seems that there may be some connection between hypertension and sodium in the diet in populations, but in the case of individuals, the evidence is equivocal.

In rats, it is possible to feed sufficient salt in the diet to cause hypertension, and certain strains of rats are far more susceptible to this treatment than others (Dahl, Heine, and Tassinari, 1962; Dahl and Shalkow, 1964). Furthermore, if the salt content of the diet is increased sufficiently, and if the treatment is started early in life, then the hypertension is self sustaining, even after the salt supplements are discontinued (Dahl and Shalkow, 1964). One would expect that since it is possible to cause hypertension by means of salt supplements in

in the diet, then it is reasonable to expect to be able to lower arterial pressure by salt restriction, but that certain individuals would be refractory to this treatment.

Quite apart from its own ability to cause hypertension, a diet high in sodium enhances the arterial pressure rise following treatment with DOC or DOCA; diets deficient in sodium prevent or reduce the rise in pressure due to these steroids (Selye and Pentz, 1943; Knowlton, Loeb, Stoerk, and Seegal, 1947; Selye, Stone, Timiras, and Schaffenburg, 1949; Danford and Herrin, 1952; Skelton, 1953). The presence of sodium in the diet appears to be essential to the development of DOC and DOCA hypertension, and the degree of hypertension attained for any dose of DOCA is dependent upon the quantity of sodium in the diet.

Surprisingly, the slight elevation of arterial pressure following upon chronic aldosterone administration is not very sensitive to the quantity of sodium in the diet (Kumar, Hall, Nakashima, and Gornall, 1955, 1957; Masson, Mikasa, and Yasuda, 1962). There is however a tendency for the blood pressure to rise higher in salt fed animals than in their normal diet mates when treated with aldosterone, though the difference is not statistically significant (Masson et al., 1962).

Considering the importance of sodium in the diet in the management of hypertensive patients, the ability of sodium itself to cause hypertension, and the striking effect which dietary sodium has upon DOC and DOCA hypertension, it is very surprising that the rise in arterial pressure due to aldosterone seems to be relatively insensitive to dietary sodium. For this reason, the action of aldosterone infusions on the arterial pressure of salt fed animals was studied. The results obtained and reported here are at variance with those in the literature showing that there is a significant difference between high, normal and low salt treated animals in their arterial pressure responses to aldosterone infusion.

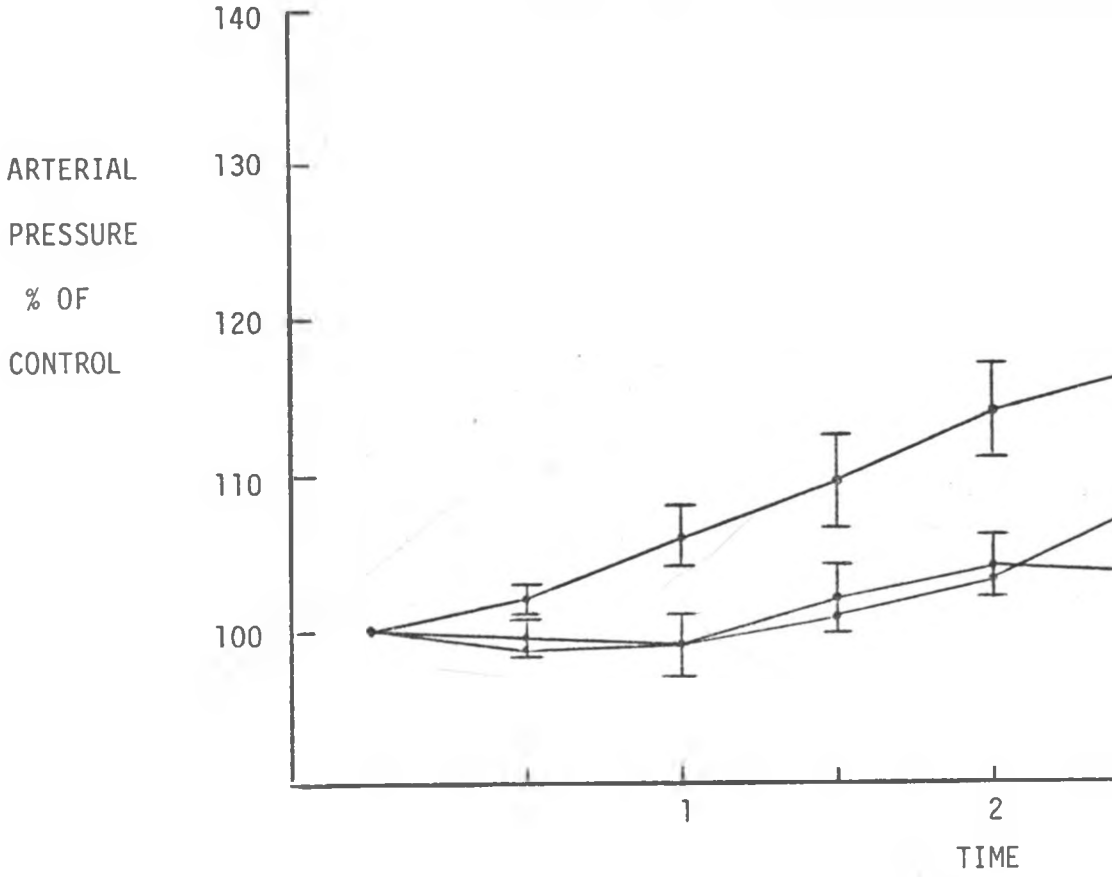
TABLE VI: Arterial blood pressures at ½ hourly intervals in high salt diet pretreated decerebrate rabbits following intravenous aldosterone infusion.

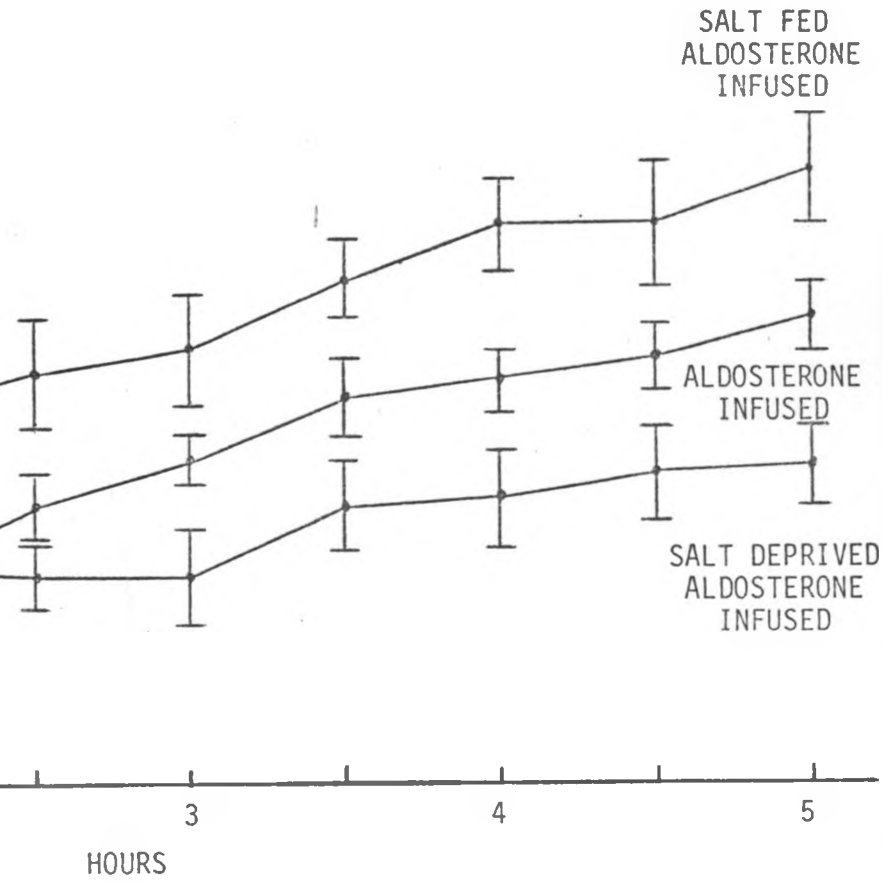
TIME	BLOOD PRESSURES										MEAN % OF STARTING PRESSURE		
											%	S.D.	S.E.
0	75	73	100	108	84	70	87	78	94	70	100		
½	77	72	100	110	84	71	88	78	98	78	102.10	3.47	1.09
1	83	74	104	110	86	70	86	84	100	84	105.80	6.45	2.04
1½	87	80	98	109	93	78	90	83	100	93	109.50	9.83	3.11
2	84	81	108	113	106	86	94	86	100	93	114.30	9.52	3.01
2½	87	80	120	120	106	89	92	88	102	95	116.40	10.61	3.35
3	87	82	124	118	108	93	92	90	104	95	118.20	11.37	3.59
3½	91	95	127	124	106	96	97	92	103	92	122.60	9.05	2.86
4	90	94	128	127	116	102	100	95	110	92	126.20	9.74	3.08
4½	85	92	130	125	116	105	101	98	112		126.11	11.87	3.95
5	90	91	132	128	119	106	104	100	120		129.55	11.09	3.69
	Mean starting pressure (mm Hg)										83.90	13.21	4.18

TABLE VII: Arterial blood pressures at ½ hourly intervals in zero salt diet pretreated decerebrate rabbits following intravenous aldosterone infusion.

TIME	BLOOD PRESSURES											MEAN % OF STARTING PRESSURE		
												%	S.D.	S.E.
0	65	71	78	64	62	58	81	75	91	69	60	100		
½	63	70	78	62	57	62	80	76	87	74	60	99.54	4.43	1.33
1	66	66	84	58	57	60	81	76	90	75	56	99.27	6.43	1.94
1½	68	69	84	58	57	61	80	75	92	72	70	101.81	7.45	2.25
2	64	72	90	64	59	63	88	74	89	75	67	104.18	6.72	2.03
2½	63	66	86	61	66	64	91	71	95	76	64	103.54	7.17	2.16
3	60	61	86	60	76	67	91	71	99	79	60	103.54	11.10	3.35
3½	66	66	90	62	78	65	92	83	100	76	60	108.09	9.39	2.83
4	69	69	94	62	79	61	96	83	105	74	59	108.36	9.58	2.89
4½	78	72	90	60	76	61	98	86	105	74	59	110.36	9.90	2.99
5	76	73	90	62	76	62	95	85	107	74	60	110.54	8.26	2.49
	Mean starting pressure (mm Hg)											70.36	10.10	3.05

FIGURE III: ALDOSTERONE INFUSION




SALT FED
ALDOSTERONE
INFUSED
ALDOSTERONE
INFUSED
SALT DEPRIVED
ALDOSTERONE
INFUSED
3
4
5
HOURS

Methods

All of the animals in these experiments were kept caged before the experiments. Ten animals were given 1% saline instead of drinking water for two weeks prior to the experiment. They drank the saline in somewhat larger amounts than the water which they had been given before, but the amount drunk was not actually measured.

Eleven animals were given a low salt regimen consisting of 10% sucrose solution replacing their drinking water, and all food was removed from their cages. They were so maintained for seven days. Although this is not the best method for achieving a salt deficient state, apart from the use of diuretics it was the only technique available here in Kenya.

The animals were prepared for the acute experiments in the same way as previous groups, and were all infused with 20 µg of aldosterone per hour contained in an infusion volume of 2.0 ml/hr.

Results

TABLES VI, VII
FIGURE III

The animals pretreated with excess salt in the diet showed a more rapid and a larger increase in arterial pressure than untreated animals. The arterial pressure began to increase right away and reached a value of 29% above the starting pressure by the end of the five hour infusion.

The animals given the low salt regimen showed very little change in pressure for the first 2 hours. Thereafter, their arterial pressure rose far less than the high salt group. After five hours of aldosterone infusion, their arterial pressure had reached only 10% above the starting value.

The mean starting pressure in the salt fed animals was 83.90 $\pm$ 4.2 which is not significantly different from untreated animals. The low salt group had a starting pressure of only

70.36 ± 3.1 which is significantly different from the untreated animals.

The control group against which these are compared is the untreated, aldosterone infused group of animals (TABLE II, FIGURE I) which is repeated for convenience in FIGURE III.

The mean starting pressure for salt fed animals was greater than that of salt fed progesterone injected animals, though the difference was not highly significant ($0.2>P>0.1$).

CHAPTER V: BARORECEPTOR

Introduction

Although it is by no means universally accepted, there is a persistent notion in the thinking of certain physiologists that the arterial baroreceptors play no role whatever in the long term regulation of blood pressure. The adherents of this view claim that the baroreceptors adapt rapidly to chronic changes in arterial pressure, and cease to try to restore the pressure to the previous normal level. "... our present concept that the baroreceptors adapt within a few days means that the baroreceptors play no role whatsoever in the control of the final equilibrium level of arterial pressure." (Guyton and Coleman, 1969). That this view is widely accepted is attested to by the fact that the above statement was made at a conference of cardiovascular scientists and was not questioned in the discussion period. Moreover, the authors did not even care to give any references to support this view, probably assuming that none would be called for.

In fact, there is little evidence supporting this dogma. In 1927, Hering showed that acute hypertension develops following bilateral section of the carotid sinus and aortic baroreceptor nerves in various animals. The production of chronic hypertension by this method was apparently not reliable. Koch and Mies (1929) produced a severe chronic hypertension in rabbits after section of these nerves, but in 1934, Koch and Mattonet were not able to repeat this and said that the hypertension they produced was only transient. In 1933, Kremer, Wright, and Scarff successfully produced chronic hypertension by this method in rabbits, but Green, de Groat, and McDonald (1935), working with both dogs and rabbits were able to produce only a transient hypertension. Boyd and McCullagh (1938), using the same techniques, but taking great care to avoid regeneration of the sectioned nerves, were also unable to produce a sustained hypertension. Nowak and Walker (1939) and

Nowak (1940) claimed to have produced successful chronic hypertension following nerve section.

The preceding catalogue of attempts to produce experimental neurogenic hypertension by section of the major baroreceptor nerves proves only that this is a very unsatisfactory method for the reliable production of chronic hypertension. A hasty conclusion which could be drawn is that the baroreceptors are not important in the long term regulation of blood pressure, and unfortunately, this is the current prevailing idea. Its opponents appear to be in disarray.

Regniers (1930), Mies (1932), and Pickering, Kissin, and Rothschild(1936) demonstrated that baroreceptor response to light pressure upon the carotid sinus was less in hypertensive individuals than in normals. More recently, sophisticated techniques have been used to show that baroreceptor adaptation does occur during the development of renal hypertension (McCubbin, Green, and Page, 1956; Peterson, Jensen, and Parnell, 1960; Kezdi, 1965; Alexander and de Cuir, 1966; Aars, 1968). Essentially, these workers have shown that in hypertension the baroreceptors lose sensitivity, sending a lower frequency of impulses to the brain for any given arterial pressure. The baroreceptors, in effect, tolerate the high blood pressure, making no attempt to lower it.

About 20 years ago, Volhard (1948), and Heymans (1952) suggested that a primary resetting of the baroreceptor might result in increased arterial pressure. The author claims that this idea is still tenable because measurement of baroreceptor sensitivity in a hypertensive individual would probably not be able to prove whether the alteration in sensitivity was primary or secondary to the appearance of the hypertension.

It is not surprising that the crude attempts to produce chronic neurogenic hypertension by sectioning baroreceptor nerves were largely unsuccessful. There are good logical reasons for this, and experiments have shown that such a procedure might not work. Undeniably, the brain is a sophisticated and versatile

computer. If one input abruptly ceases to function, and therefore disagrees with the remaining functioning inputs, the brain is likely to ignore the malfunction and rely upon the working ones. Ead, Green, and Neil (1952) showed that this is, in fact, true for the baroreceptors. They arranged to alter quickly the input pressure to the carotid sinus from pulsatile to steady, and back again, while maintaining the mean pressure constant. They found that pulsatile pressure stimulates the carotid sinus to produce a higher mean output frequency than does a steady flow of the same mean pressure, but the systemic pressure nevertheless remained unchanged. However, when the aortic depressor nerves were cut, the change from pulsatile flow to steady flow in the carotid sinus resulted in a rise in systemic pressure - presumably because in the absence of normally functioning aortic baroreceptors, the brain was forced to rely entirely upon the carotid baroreceptors, and now, a change in their mean output frequency resulted in a change in systemic arterial pressure. It is, therefore, not inconceivable that when the main baroreceptor areas are inactivated, so that they send a zero signal to the brain, the brain will soon learn to ignore them, especially if there is a normally functioning baroreceptor remaining.

In order to test the idea proposed by Volhard, and by Heymans, that it is possible to induce a change in arterial pressure by means of a primary resetting of the baroreceptor, it is necessary to alter the baroreceptor in such a way that the normal pattern of discharge is preserved. If this is done carefully, then the brain will not be able to detect that the baroreceptor is malfunctioning and delivering a spurious output. To do this, the carotid sinus was embedded in a rapidly setting epoxy resin, while the internal carotid artery was clamped. The result of this operation was that the epoxy cast prevented the expansion of the carotid artery when it was refilled with blood, and hence chronically reduced the discharge frequency from the baroreceptor. Presumably, some degree of pulsation would still be detected by the baroreceptor, but the

mean pressure registered by the carotid baroreceptor would be somewhat reduced, as would the extent of pulsation. A baroreceptor modified in this way should still be able to signal a decrease in arterial pressure normally, and testing showed that this was indeed the case.

While the experiments with aldosterone infusions were being performed, it was noted that the heart rate throughout altered very little despite the rising arterial pressure. It was a little surprising, because typically, the heart rate would show some reduction in the face of the pressure increase. The only conclusion which could be drawn from this is that the baroreceptors were making no effort to decrease the arterial pressure during the aldosterone infusion - or, at least were making no effort to lower pressure by means of heart rate reduction.

A group of experiments were performed, in which an attempt was made to raise arterial pressure to the same levels achieved in the aldosterone infusions by means of angiotensin infusions, and by noradrenaline infusions. Surprisingly enough, these experiments did not succeed. It was not possible to maintain a chronically elevated arterial pressure by the infusion of vasoconstrictors. At the same time, it was noted that the heart rate decreased substantially, suggesting that the baroreceptors were powerfully opposing the attempt to increase arterial pressure.

In experimenting with decerebrate animals, it was obvious that baroreceptor reflexes in these preparations were far more lively than those seen in anaesthetised animals. An attempt to study systematically the ability to raise arterial pressure by means of a vasoconstrictor agent (noradrenaline) in decerebrate animals, before and after anaesthesia with nembutal is reported here. It is clear that barbiturate anaesthesia attenuates the baroreceptor responses, and also permits noradrenaline to cause a far greater rise in arterial pressure than in the unanaesthetised preparation with sensitive baroreceptor reflexes.

In an attempt to explain the rapid and large rise in arterial pressure achieved by aldosterone infusions, when infusion of vasoconstrictor substances failed to do likewise, the action of aldosterone on the carotid sinus baroreceptor was investigated. In initial experiments, aldosterone in high concentration was merely applied to the outside of the bifurcation of the carotid artery, and its effects noted, and compared to the effects of a similar saline application, and to intravenous infusions of the same quantities of aldosterone.

Later experiments were more sophisticated. The carotid sinuses of one animal were exposed, isolated from the rest of the circulation, and perfused with blood from another animal. The second animal was then infused with aldosterone, and the blood pressures of both were monitored. It was found possible to raise the blood pressure of the perfused animal, which could only have happened if the baroreceptor itself was sensitive to the hormone, as it did not enter the circulatory system of that animal. Furthermore, it was found that infusion of aldosterone into the perfused animal were ineffective, since its carotid sinuses were protected from the action of aldosterone, being supplied with relatively aldosterone free blood from the donor animal.

A) Carotid Sinus Encapsulation

Methods

Rabbits of either sex were given 50 µg of atropine sulphate (Antigen) and anaesthetised with pentobarbitone (Nembutal). The animals were then carefully shaved about the neck, and thoroughly swabbed with savlon. From this point, antiseptic and sterile precautions were observed as carefully as possible.

The carotid arteries were carefully exposed in the region of the carotid sinus, taking extreme care not to damage the carotid sinus and aortic nerves. The wound area was then swabbed with savlon, and dried. The carotid artery was clamped with a bulldog clamp. A small quantity of ultra rapid hardening

epoxy resin (S.S. White, "Hardset" Dental Impression Paste) was then injected around the carotid sinus from a 2 cc syringe and a 19 gauge needle suitably bent and blunted. Care was taken to ensure that the epoxy completely surrounded the carotid sinus. When the cast was completely hard, the arterial clamp was removed. The procedure was then repeated on the other carotid artery, the animal sewn up, injected with antibiotic, and allowed to recover.

During the recovery period, the animal cages were cleaned more frequently than normal, and the animals received daily injections of antibiotic (Seclomycin) for about seven days.

The experimental animals had their arterial pressures measured daily for two weeks prior to the surgery, and for approximately 50 days after surgery, using the ear capsule method (Grant and Rothschild, 1934).

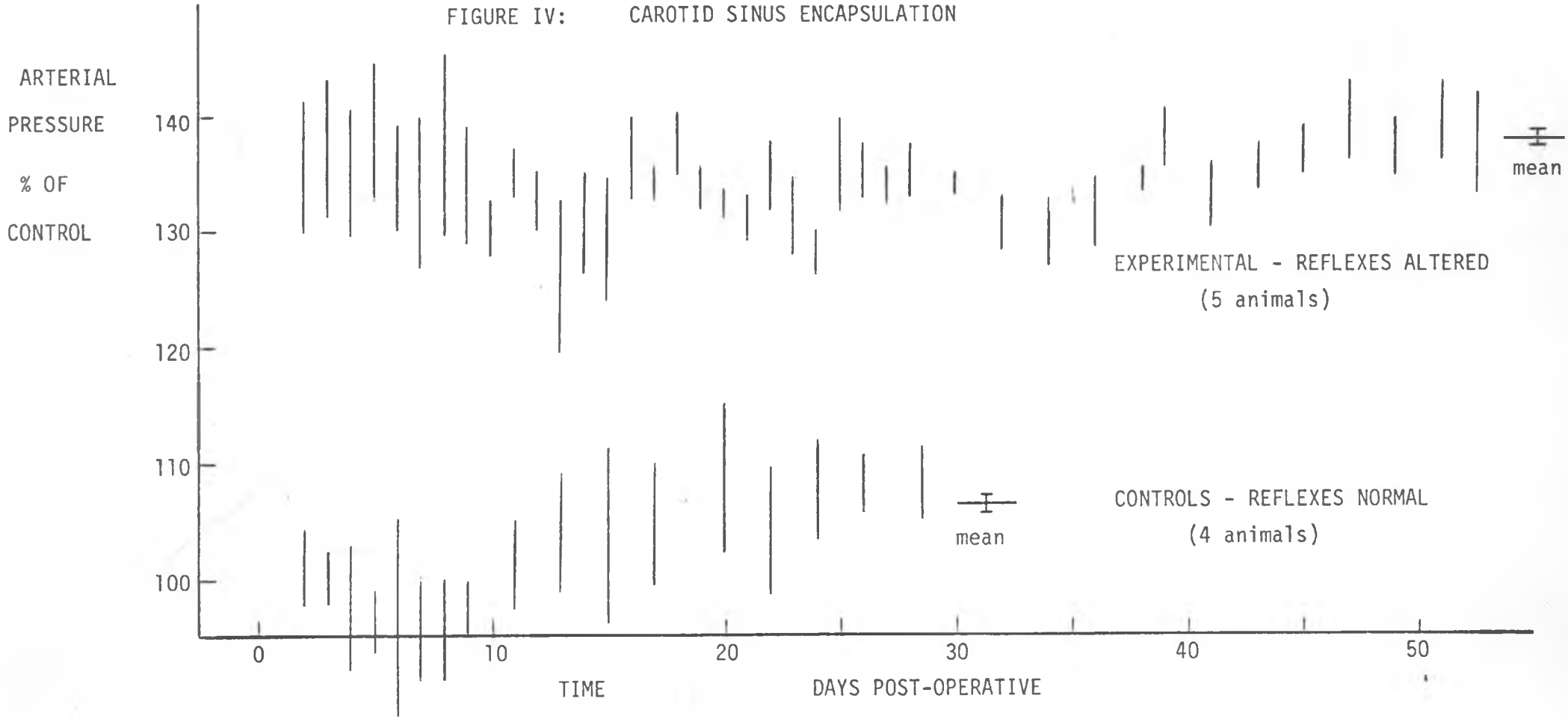
After the experimental period of 50 days was over, the animals were decerebrated as described in CHAPTER II, and their vasomotor reflexes were tested. While observing the arterial blood pressure, the carotid arteries were bilaterally clamped. Then, angiotensin was injected in sufficient amount to cause a rise in arterial pressure of about 30 mm Hg. In both cases, the response of the baroreceptors to the increased or decreased pressure in the carotid sinus was noted from the effects upon arterial pressure and heart rate.

Results FIGURE IV

The mortality rate of the animals in the experiments was unfortunately high, as a result of infections which were refractory to the antibiotics we had available.

The animals were not classified and grouped until the end of the experiment. The animals were placed into groups depending upon the degree and type of interference which the cast produced upon the baroreceptor reflexes.

FIGURE IV: CAROTID SINUS ENCAPSULATION



The first group of animals were those whose baroreceptor reflexes were incapable of detecting an increased pressure, but which responded to low pressure normally. These were the animals in which the cast had had the desired effect, and these five animals were labelled A.

The second group of animals were those which exhibited no baroreceptor reflexes at all so that it appeared as if the carotid sinus nerves had been cut. However, one of these showed a reflex similar to Group A in only one carotid. These three animals were labelled B.

The third group of animals were those in which the cast had had no effect upon the baroreceptor reflexes at all, and indeed, the cast had been purposefully placed away from the sinus region in two of these. They responded to both high and low arterial pressure equally well. These four animals were labelled C.

The animals in Group A showed an immediate increase in arterial pressure from the first day. They maintained the approximately 38% increase throughout the duration of the fifty days during which the arterial pressure was taken.

The animals in Group B each behaved differently. One animal showed no change in arterial pressure at all, and died at 18 days (baroreceptor reflex was tested when it was in poor health on the last day). One animal showed unchanged pressure for six days, and thereafter a small rise (varying between 7% and 14%) which was maintained for the duration of the experiment. The last animal showed an immediate rise in pressure to 60% above control which declined by the fourth day to a steady level of 18% to 28% above control for the duration of the experiment. This was the animal with a baro-receptor reflex (properly interfered with) in one carotid artery only.

The animals in Group C showed no clearcut rise in arterial pressure at all. They served as controls for the effects of

TABLE VIII: Noradrenaline infusions into decerebrate rabbits first without then with nembutal anaesthesia.

Time Minutes	Arterial Pressures									% of Control I Mean	S.D.	S.E.	% Rise in Pressure Following Bilateral Carotid Occlusion
	CONTROL I												
0	100	85	95	80	88	80	90	100	120				
30	102	80	96	80	92	94	96	84	118	100			30.2%
	NORADRENALINE INFUSION 1 mg/kg/hr												
2	136	120	144	132	138	134	160	120	160	148.6	11.6	3.9	
10	120	100	100	106	106	118	110	86	110	114.4	12.7	4.2	
20	115	85	100	104	100	114	104	84	110	104.3	11.0	3.7	
30	110	85	110	102	98	114	106	88	110	110.0	9.8	3.3	15.0%
	NORADRENALINE INFUSION 2 mg/kg/hr												
2				140	116	136	166	132	156	151.3	20.6	8.4	
10				132	100	124	140	120	130	134.1	21.9	9.0	
20				122	108	120	140	112	136	132.6	15.3	6.3	
30				120	108	120	138	116	142	131.8	13.1	5.3	9.3%
	NEMBUTAL 4.0 mg/kg/hr NORADRENALINE INFUSION 2 mg/kg/hr												
10				106	130	108	126	86	130	121.8	15.1	6.2	
20			108	110	120	110	130	110	144	129.0	8.0	3.3	
30	110	90	120	108	116	112	136	116	160	132.5	8.5	3.5	4.4%

			INFUSION OFF		CONTROL II				
10	70	60		48	40	60	44	44	40
20	66	60	48	46	48	58	62	48	70
30	64	60	55	44	50	58	70	50	70
			NORADRENALINE INFUSION 1 mg/kg/hr						
2			140	74	120	90	150	124	144
10			128	74	100	82	118	112	120
20			124	76	90	86	120	110	124
30			120	76	90	90	124	112	130
			NORADRENALINE INFUSION 2 mg/kg/hr						
2				80	130	106	160	156	180
10				88	110	100	150	140	180
20				88	110	98	145	130	180
30				88	110	98	150	125	180
			INFUSION OFF						
10						80	76	40	60
20						60	72	40	60

49.8	11.1	4.6	
58.8	4.5	1.9	
60.7	6.8	2.8	11.7%
% of Control II			
211.6	39.7	15.0	
186.1	33.8	12.8	
184.4	28.0	10.6	
187.0	25.3	9.6	
237.0	51.3	21.1	
223.5	40.2	16.5	
218.7	36.2	14.8	
218.0	33.9	13.9	
103.3	26.3	13.2	
93.0	11.8	5.9	

the cast material on the body (toxicity) and for the effects of supposedly interfering with the blood flow to the brain.

It was noted that in some of the animals, the epoxy cast had been absorbed, and replaced by fibrous scar tissue. However, the criterion of whether the desired effect had been achieved upon the baroreceptor reflexes did not depend upon the appearance of the cast, but the physiological test of baroreceptor performance.

B) Noradrenaline Infusion

Methods

Animals were prepared as described in CHAPTER II (Methods) for the recording of arterial pressure, and the receipt of an infusion.

Arterial pressure was recorded for one hour after decerebration. During this time, the baroreceptor reflexes were tested by means of carotid artery compression for two minutes. Noradrenaline was infused at the rate of 1 mg/kg/hr, contained in 2 ml/hr of saline. The infusion was continued for 30 minutes, and the baroreceptor reflexes were tested again during this period. At the end of thirty minutes, the rate of infusion was doubled for a further thirty minutes. After one hour of noradrenaline infusion, the infusion still continuing, an anaesthetic dose of nembutal was given intravenously over five minutes. At the end of a further 30 minutes, (total time of infusion being 90 minutes) the infusion was stopped.

A 30 minute control period without infusion was allowed, and then the noradrenaline infusion was restarted at the rate of 1 mg/kg/hr for thirty minutes, and then doubled for a further thirty minutes.

Results

TABLE VIII, FIGURES V, VI

Although the arterial pressure rose rapidly at the commencement of the infusion, it soon declined, so that after

FIGURE V: NORADRENALINE INFUSION

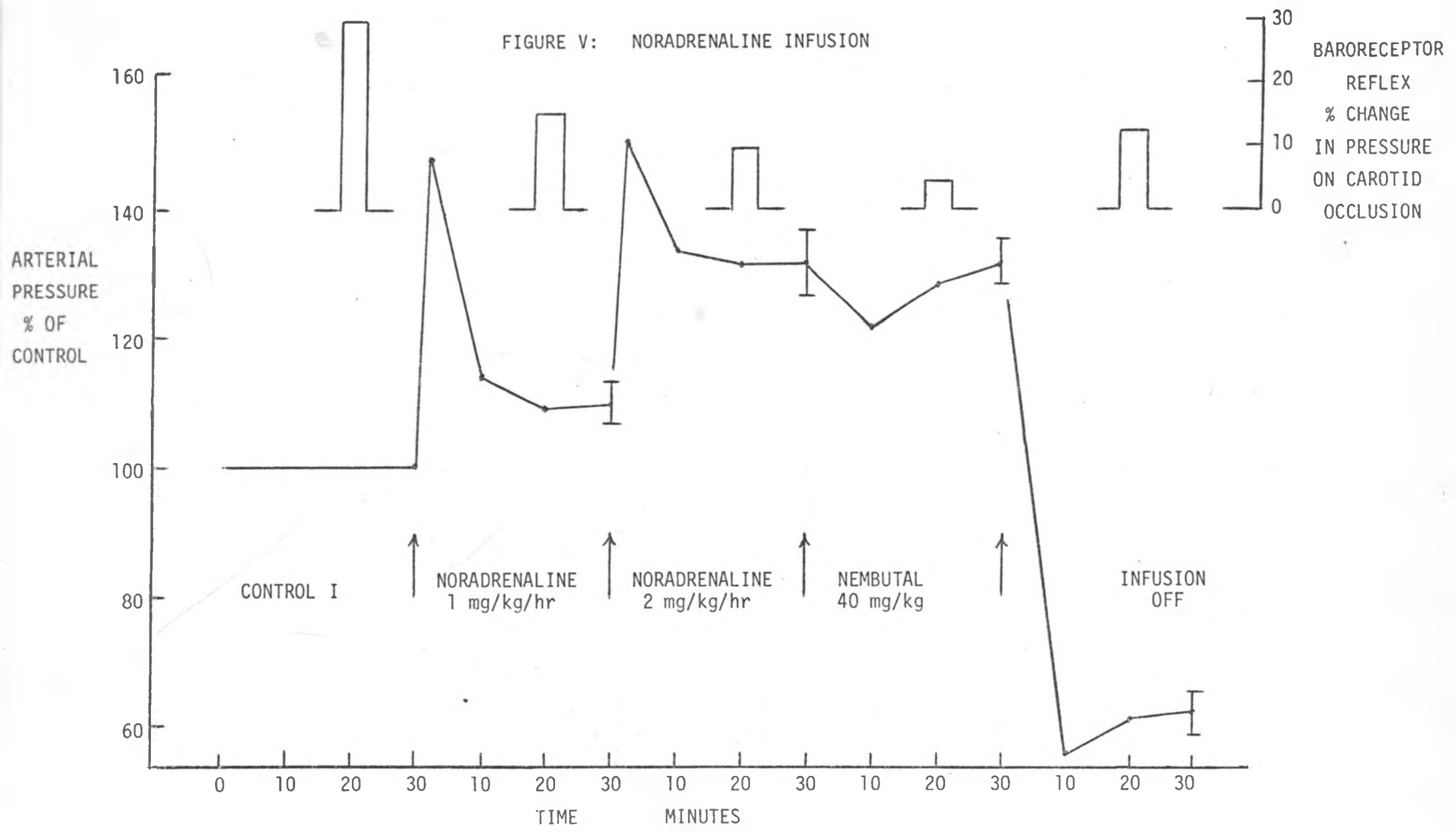
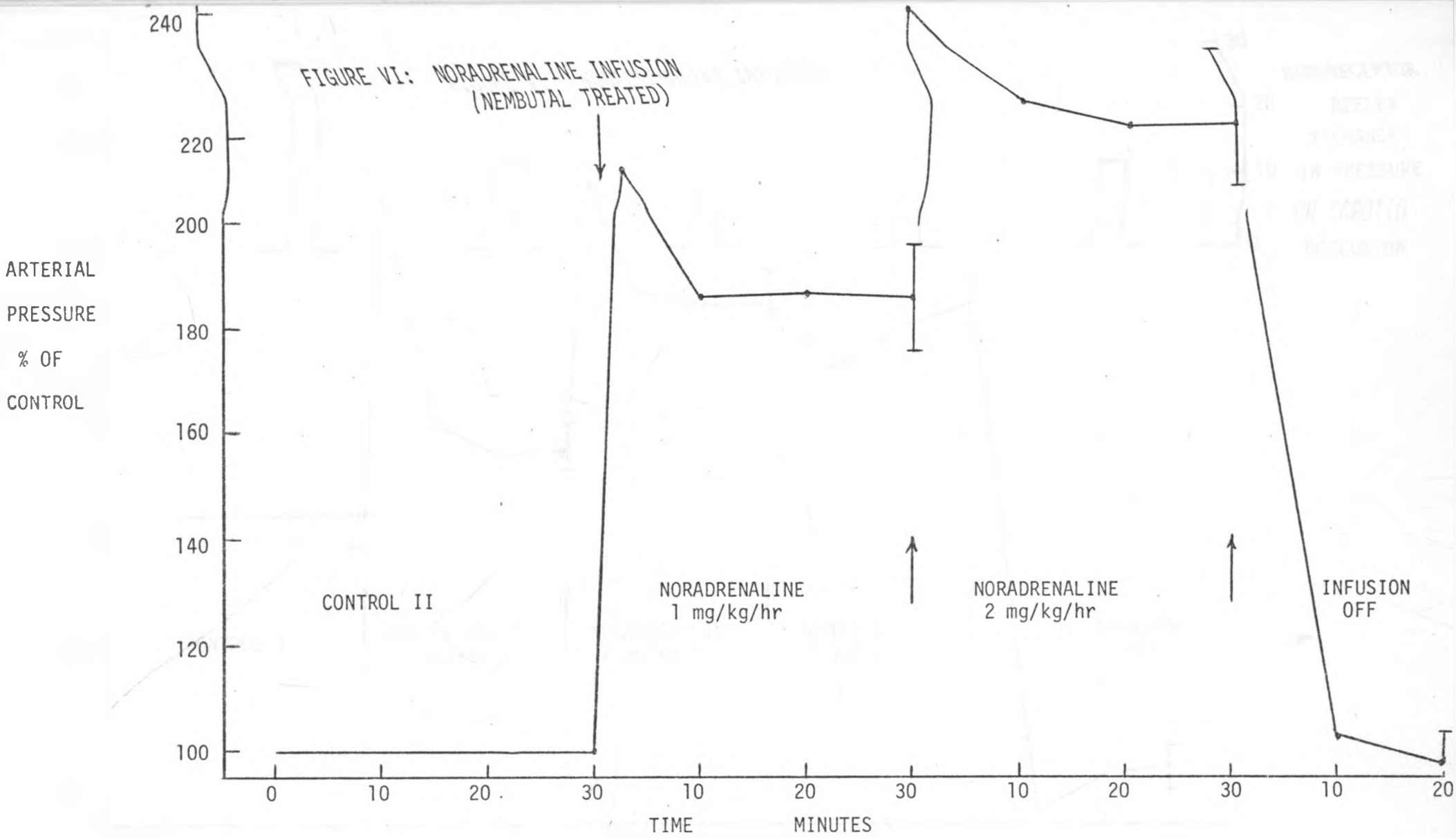


FIGURE VI: NORADRENALINE INFUSION
(NEMBUTAL TREATED)



30 minutes it was only 10% above control. Increasing the infusion rate caused the pressure to peak once again, and subside, but to 32% above control this time. Administration of nembutal at this time caused the arterial pressure to drop transiently, but again to attain the level of 32% over the uninfused control level. Thus in the infused animal, nembutal caused no change in the arterial pressure.

When the infusion was stopped, the arterial pressure fell very rapidly to a level roughly equal to 62% of the uninfused, unanaesthetised control. Thus, nembutal appeared to lower considerably the arterial pressure of decerebrate animals.

Using the uninfused, but anaesthetised, arterial pressure as a new control level, restarting the infusion resulted in a very large increase in the arterial pressure, which stabilised at 87% above the control level. Doubling the infusion rate at this point resulted in a further increase in arterial pressure which stabilised at 118% above control.

The baroreceptor reflexes declined to less than 1/2 the control values after the nembutal injection.

C) Aldosterone Superfusion of Carotid Sinus

Methods

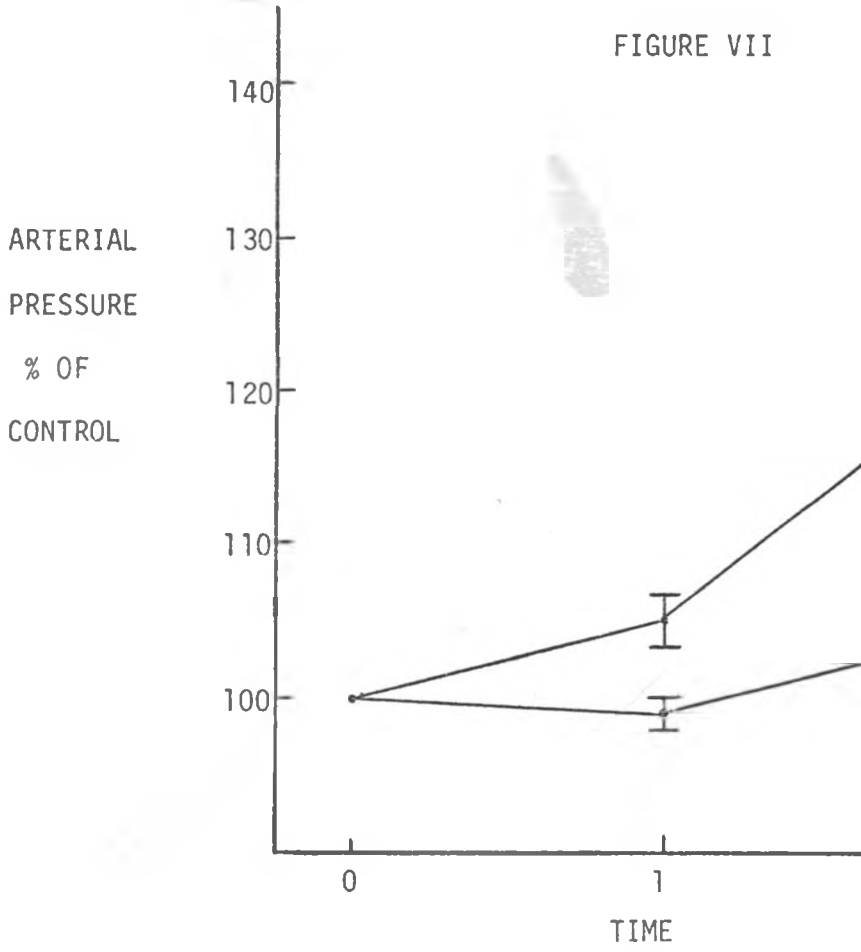
Animals were prepared as in the preceding experiments. Four animals were given infusions of 100 µg/hr aldosterone in saline (50 µg/ml) intravenously.

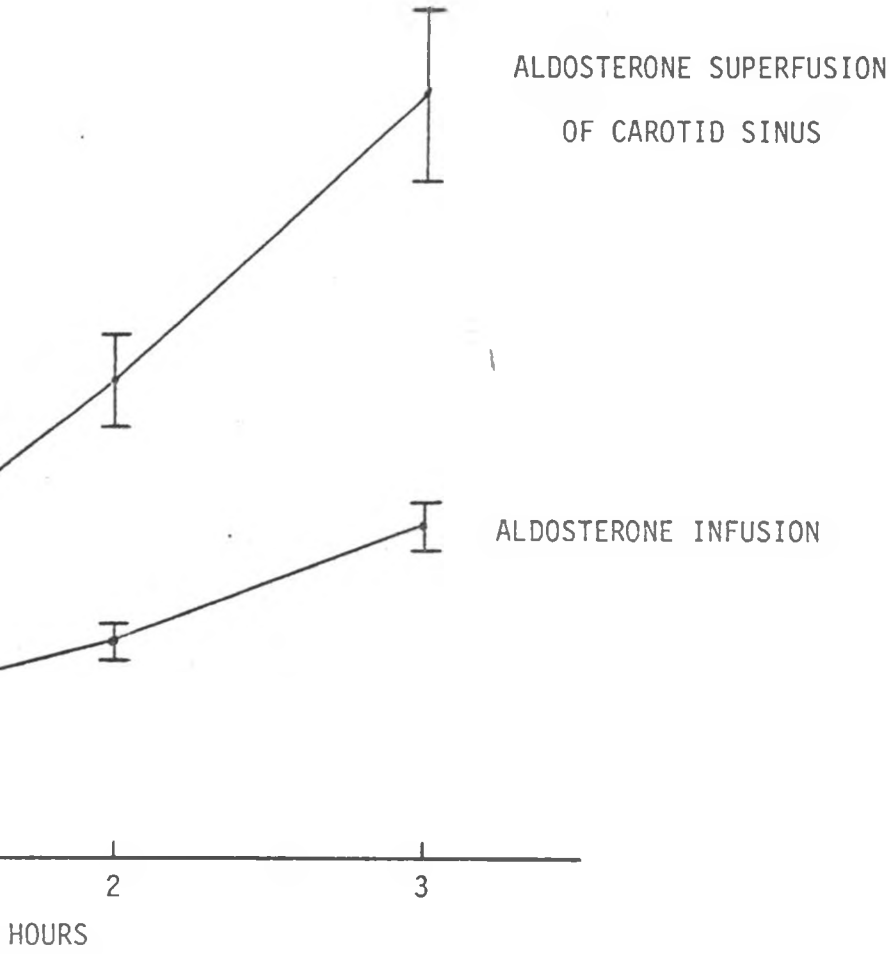
Six animals were prepared similarly, but in addition, the carotid sinus region was exposed in the neck, and fine needles were applied to the outside of each sinus and connected to the infusion pump via fine catheters. In these animals a saline superfusion was given to the sinus region at the rate of 2 ml/hr for one hour. This was then changed over to a solution containing 50 µg/ml (100 µg/hr) aldosterone which was administered to the sinus region for three hours.

TABLE IX: Arterial pressures at hourly intervals in decerebrate rabbits following either aldosterone superfusion of carotid sinuses or intravenous infusion of similar quantities of aldosterone.

TIME	BLOOD PRESSURES				% OF CONTROL MEAN	S.D.	S.E.
ALDOSTERONE: INTRAVENOUS INFUSION 100 ug/hr							
-1	99	87	102	75	} 100		
0	101	88	102	75			
1	100	88	99	72	98.75	1.89	0.94
2	106	92	101	77	103.75	2.21	1.10
3	111	102	111	87	111.50	3.31	1.65
ALDOSTERONE: SUPERFUSION OF SINUS 100 ug/hr							
-1	80	81	80	66	75	71	} 100
0	82	79	83	63	78	70	
1	91	87	80	65	83	76	104.83	4.35	1.78
2	100	109	97	74	89	82	121.33	7.68	3.14
3	109	127	125	88	99	87	139.66	13.95	5.71

FIGURE VII




ALDOSTERONE SUPERFUSION
OF CAROTID SINUS
ALDOSTERONE INFUSION
2
3
HOURS

In all cases, the infusion or superfusion was not begun until after the blood pressure had remained stable for 30 minutes which usually occurred one hour after the decerebration.

Results

TABLE IX

FIGURE VII

In the animals given the intravenous infusion of aldosterone, the rate of rise in arterial pressure was the same as that seen in the previous experiments using smaller amounts of the hormone. The pressure did not rise at all until about 1 1/2 hours. After three hours, the pressure rise seen was 11%.

The animals given the aldosterone superfusion showed a pressure rise of 5% in the first hour, and thereafter, a very steep rise reaching 40% in three hours.

It is to be noted that the superfusion of aldosterone acted more rapidly and had a larger total effect than the intravenous infusion of the same amount.

D) Aldosterone Perfusion of Carotid Sinus by Cross-Circulation

Methods

In each experiment, two rabbits were used. One was decerebrated as described before, and the other was anaesthetised with sodium pentobarbitone (Nembutal, Abbott). Both animals were mechanically respired.

The pentobarbitone anaesthetised animal was prepared with plastic cannulae in both femoral arteries, and both femoral veins, and one cannula in a jugular vein. These were called the donor animals.

The decerebrate animal was prepared so as to have both carotid arteries, before and beyond the carotid sinus

TABLES X, XI, XII: Cross-Circulation: Anaesthetised donor perfusing the carotid sinuses of decerebrate recipient. Arterial blood pressures in either donor or recipient at half hourly intervals following intravenous aldosterone infusion into either donor or recipient.

TABLE X: Aldosterone into donor, arterial pressures of donor.

TIME	BLOOD PRESSURES					% OF CONTROL MEAN	S.D.	S.E.
-1	64	48	75	72	60	100		
-½	64	48	80	80	59			
0	70	48	80	74	59			
½	70	48	78	85	60	102.00	4.64	2.10
1	72	44	80	90	58	102.00	9.30	4.17
1½	72	49	81	84	56	102.00	5.00	2.24
2		50	79	91	61	106.00	8.28	4.14
2½		49	82	85	58	103.00	5.03	2.51
3		47	82	87	58	102.75	2.08	3.54
Mean starting pressure (mm Hg)						66.80	13.25	5.94

TABLE XI: Aldosterone into donor, recipient arterial pressures.

TIME	BLOOD PRESSURES					% OF CONTROL MEAN	S.D.	S.E.
-1	76	112	69	72	80	100		
-½	72	108	61	65	87			
0	75	108	62	60	86			
½	98	108	68	62	78	107.00	15.04	6.74
1	100	110	80	67	90	116.20	14.06	6.30
1½	106	113	84	81	95	125.60	16.36	7.33
2		120	92	87	98	129.50	19.70	9.85
2½		128	90	95	94	133.00	22.31	11.15
3		130	94	98	100	138.25	22.73	11.36
Mean starting pressure (mm Hg)						78.20	19.70	8.83

TABLE XII: Aldosterone into recipient, arterial pressures of recipient.

TIME	BLOOD PRESSURES					% OF CONTROL MEAN	S.D.	S.E.
-1	87	82	105	66	60			
-½	100	97	109	74	65	100		
0	98	100	119	68	59			
½	88	110	112	66	61	99.20	7.66	3.43
1	88	104	110	64	63	97.60	6.80	3.04
1½	87	96	103	67	61	94.80	5.76	2.58
2	84	106	107	66	59	95.80	7.66	3.43
2½	77	106	109	64	66	96.60	12.48	5.59
3	85	99	102	60	56	91.00	5.14	2.30
	Mean starting pressure (mm Hg)					88.40	23.33	10.46

completely isolated from the animal's circulation. These were called the recipient animals. All but one branch of the carotid beyond the sinus was tied, and the remaining branch was cannulated back towards the sinus. The common carotid was then tied off, and cannulated above the tie (again towards the sinus). This large cannula received blood from one of the femoral arteries of the donor animal prepared simultaneously, and the other cannula, above the sinus delivered the blood back to the donor animal via the femoral vein. This was a small diameter cannula so as to provide an artificial resistance to flow. When both carotid sinus regions were being perfused with blood from the donor animal, then the vasomotor reflexes of the recipient animal were tested by lowering the blood pressure in the sinus (stopping the flow temporarily) and by raising the perfusion pressure (injection of angiotensin into the donor animal). The blood pressures of both animals were recorded on the same moving paper, and could be easily watched. When the pressure in the donor animal rose, then the pressure in the recipient fell, and vice versa. If the reflexes were no longer functioning, due to damage of the sinus, then the experiment was regretfully discarded.

In five experiments the donor animal was infused with 20 µg/hr of aldosterone, and in five experiments, the recipient animal was infused with 20 µg/hr of aldosterone.

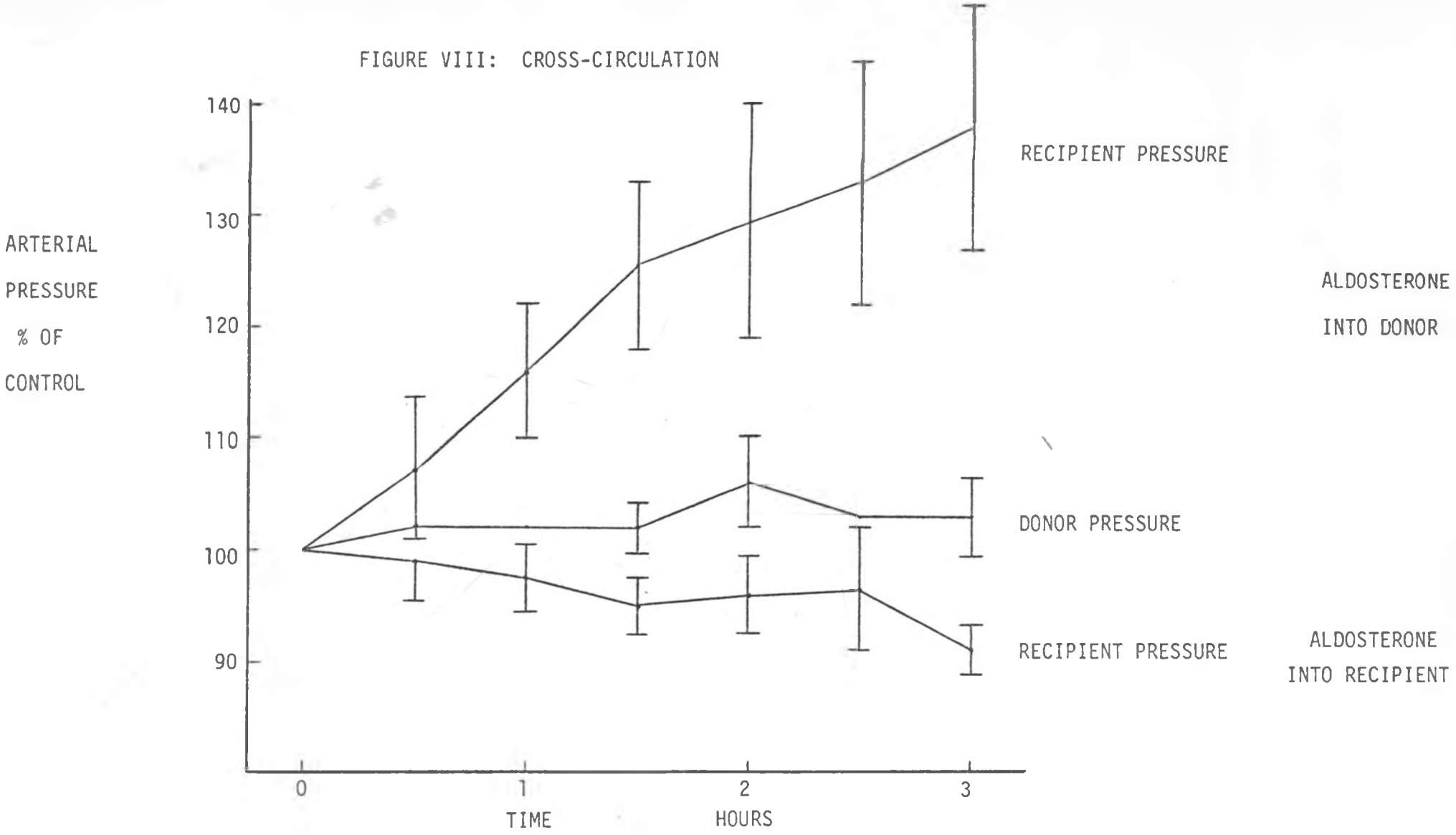
Results

TABLES X, XI, XII
FIGURE VIII

The blood pressure in the donor animal remained very stable throughout all of the experiments. This was probably due to the anaesthetic which tends to suppress vasomotor reflexes resulting in a stable pressure.

When the donor animal was infused with aldosterone at the rate of 20 µg/hr, then the blood pressure of the recipient

FIGURE VIII: CROSS-CIRCULATION



began an immediate rise, reaching 38% above the mean starting pressure in three hours. The speed and intensity of the rise were very remarkable, especially as the donor animal pressure tended to rise somewhat rather than fall during the same time which would normally have produced a reflex fall in the donor animal.

When the recipient animal was infused with aldosterone at the rate of 20 µg/hr, then surprisingly, the pressure fell somewhat, reaching 91% of its starting pressure in three hours.

CHAPTER VI: CHOLESTEROL

Introduction

Cholesterol has long been implicated as a causative factor in human cardiovascular disease. In this laboratory, attention was directed to cholesterol because it is chemically related to the steroid hormones, and might have similar effects. The presence of cholesterol in the bloodstream in large and, in any one individual, apparently fairly constant amounts (Bhattathiry and Siperstein, 1963) is something of a mystery. High levels of cholesterol in the blood are definitely associated with increased risk of cardiovascular disease (Dawber, Kannel, Revotskie, and Ragan, 1962; Karvonen, 1962; Leren, 1966, 1968, 1970). Vascular lesions can be caused in experimental animals by simply raising the blood cholesterol to very high levels (reviewed in Mitchell and Schwartz, 1965). It has been in the area of arterial disease that most of the experimentation with cholesterol has been centered to date.

So far there has been little interest, and hence little evidence for an effect of arterial blood pressure upon blood cholesterol concentration. Daly, Deming, Raeff, and Brun (1963), however, showed that renal hypertension in rats tended to raise the plasma cholesterol concentration, but offered no explanation for this. For over fifty years, there has been slight, but steady interest in the effects of blood cholesterol elevation upon the arterial pressure. The majority of investigators agree that increased blood cholesterol, induced by a special cholesterologenic diet fed over many weeks, is associated with increases in the arterial pressure (Fahr, 1912; Schmidtmann, 1925; Thomas, 1926; Kuntz and Sulkin, 1949; Bronte-Stewart and Heptinstall, 1954; Whittington-Coleman, Carrier, and Clower, 1968). Others reported a rise in pressure in only some of their animals, or no change at all (Deiche, 1926; Dominguez, 1927; Scarff, 1927; Schmidtmann and Huttich, 1928; Katz, Sanders, Megibow, and Carlen,

1940). In most of these studies, the blood cholesterol was raised between 5 and 30 times above the normal.

Since the rise in arterial pressure seen in the experimental animals on high cholesterol diets was both relatively slight, and somewhat uncertain, the lack of interest in cholesterol as a causative agent in human hypertension is understandable. However, it is interesting to note here some strange experiments performed over 20 years ago. Kellner, Correll, and Ladd (1949, 1951) chronically administered intravenous injections of detergents to rabbits. They noted that the blood cholesterol concentration in these animals rose to very high levels but that the incidence of atherosclerotic placques was lower than in normal animals. Animals on a high cholesterol diet showed a significant reduction in arterial lesions after detergent injections. The authors explained their findings by suggesting that the simultaneous rise in blood phospholipid concentration was responsible for protecting the animals against the development of arterial disease. Another possible but highly speculative explanation, which is favoured by this author is based on the presence of a feedback loop controlling cholesterol concentration in the bloodstream. Hepatic synthesis of cholesterol is adjusted to the daily intake of the steroid, presumably to maintain a constant concentration in the bloodstream (Bhattathiry and Siperstein, 1963). In the experiments of Kellner et al. (1949, 1951), the detergent injections probably bound much of the circulating cholesterol, thereby reducing its effective concentration. The animal then probably accelerated its rate of synthesis in an attempt to make up the deficit, only to have the cholesterol continuously "mopped up" by the daily detergent injections. The net result of this treatment was to raise the biochemically assayable blood cholesterol concentration, but to lower it as far as the body was able to detect - as evidenced by the lowered incidence of atherosclerotic placques.

It is generally agreed that raised blood cholesterol levels increase the incidence and severity of atherosclerotic placques in the arteries (reviewed by H. Selye in Experimental Cardiovascular Diseases, 1970). However, these odd experiments suggest that it is not only the quantity of cholesterol which matters, but its form and/or binding in the bloodstream as well.

Human cardiovascular disease is associated with elevated blood cholesterol concentrations, but these are rarely higher than double the normal (Dawber et al., 1962; Leren, 1966, 1968, 1970). Even in familial hypercholesterolemia, where blood cholesterol concentrations are very high indeed and, incidentally, the cholesterol feedback control no longer operates, they rarely exceed four times the normal (Khachadurian, 1969). Thus, if one is to implicate cholesterol experimentally as a contributory factor to arterial disease, or hypertension, one must limit the experimental increase in blood concentration so that it remains within the range seen in human disease.

Accordingly, acute infusions of cholesterol in quite small amounts were given to test the short term effects on arterial pressure. Also, an attempt was made to test the effect of chronic high blood concentrations of cholesterol on rabbits. These experiments failed, because it was found impossible to raise their blood cholesterol merely by increasing the amount of saturated fat in their diet. However, the high fat diet was accompanied by a marked increase in the arterial pressure, indicating that other agents in a high fat diet might be responsible for hypertension.

Finally, yet another possible action of cholesterol must be considered. It is no longer in dispute that high cholesterol diets greatly increase the probability and severity of atherosclerotic lesions. These lesions occur in the major arteries, and the aorta and carotid bifurcation are favourite locations. In the event of an atherosclerotic placque forming upon the arterial wall of a baroreceptor region, the mechanical

properties of the baroreceptor, and hence its sensitivity would be altered. In the DISCUSSION, the importance of the baroreceptors in the generation and prevention of hypertension will be discussed. It should be kept in mind that atherosclerosis, or "hardening of the arteries" may have a profound desensitising effect upon baroreceptors .

Methods

Animals were prepared as in CHAPTER II, for the recording of arterial pressure and receipt of an infusion.

Cholesterol (BDH) was infused as an emulsion in saline. The emulsion was prepared by the ultrasonic emulsification of cholesterol powder in water. Cholesterol powder was mixed with a very small amount of water to form a thick paste - much like the mixing of cocoa powder. The paste was then diluted with distilled water, placed into a 20 ml glass bottle, and then tightly stoppered onto the probe of the ultrasonic power source (Branson Sonic Power) carefully excluding any air from the bottle. The mix was "sonified" at 100-150 watts power for 5-10 minutes, while cooled with an ice water bath. This technique was suggested by the company (Branson Sonic Power) whose expert claimed that sonication of this duration should not result in any derangement of the cholesterol molecule. In addition, a sample of the cholesterol emulsion was sent to Prof. J. Landon of St. Bartholomew's Hospital, London, for analysis in a mass spectrometer. He reports that no impurities or degradation products were detectable, and that the sample "appeared to be pure cholesterol". This procedure was repeated many times until about 250 ml of cholesterol emulsion was obtained. The emulsion was centrifuged for about 5 minutes in a standard blood centrifuge, and the supernatant collected. The supernatant was then filtered through a 5 μ millipore filter ensuring that the infusion contained no particles larger than this. This filtered emulsion in distilled water was both assayed by a standard

colorimetric assay (Henly's Method for Total Cholesterol, in "Practical Clinical Biochemistry", Heinemann), and evaporated to dryness and weighed. The cholesterol concentration determined by these two methods was 6.5 mg/ml.

Immediately prior to performing each experiment, the stock emulsion was diluted with an amount of saline so that the infusion would deliver 0.3 mg/kg/hr in a volume of 2.0 ml/hr. It was noted that for the entire period of time over which these experiments were performed, the stock emulsion showed no signs of precipitation. A total of 14 animals were infused with the cholesterol emulsion, though, due to a rash of power failures, only 6 survived four hours or longer.

Control experiments were performed in exactly the same way except that talc, treated in the same way as the cholesterol, was used instead. The talc was assayed only by drying and weighing. The infusion rate for the talc was exactly the same as that for the cholesterol. A total of 8 animals were infused with the talc emulsion.

A second series of cholesterol experiments was carried out using rabbit plasma containing protein bound cholesterol. Five rabbits were exsanguinated under nembutal anaesthesia, and the blood collected in citrated containers. The blood was centrifuged, and the plasma drawn off. The plasma was then dialysed against saline for three days in the cold to remove the citrate, anaesthetic, catecholamines, and hopefully the polypeptides as well. The dialysate was filtered through the 5 μ filter to remove particles and plasma clots, and assayed for cholesterol. The concentration of cholesterol in the dialysed plasma was found to be about 3.5 mg/ml, and before infusion, it was diluted so that the infusion delivered 0.3 mg/kg/hr in a total volume of 2.0 ml/hr. Six experiments were performed using this protein bound cholesterol, and then the plasma dialysate was dialysed once again for three days in the cold. It was filtered again through the

TABLE XIII: Arterial pressures at half hourly intervals in decerebrate rabbits following intravenous infusion of cholesterol emulsion.

TIME	BLOOD PRESSURES														% OF CONTROL MEAN	S.D.	S.E.
-1	95	68	70	80	92	78	75		68	88	90	80	68	62			
-½	100	66	70	75	78	76	70	80	68	84	86	78	76	60	100		
0	99	60	68	64	81	72	75	78	76	88	86	74	80	60			
½	104	58	76	70	88	80	80	80	78	91	92	74	86	70	107.28	5.20	1.39
1	112	62	76	84	100	88	88	90	78	95	93	88	86	74	117.50	7.65	2.04
1½	116	68	80	88	100	90	96	94	79	100	100	98	90	70	120.92	7.97	2.13
2	115	75	86	95	105	96	97	99	80	108	102	105	98	75	127.50	9.17	2.45
2½	113			98	101	97	98	92	88	110	104	100	98	78	128.16	9.87	2.85
3	120			105	99	100	98	95	100	110	110	95	96	82	132.08	11.67	3.37
3½	125			104		100	96	90		112	120		100	86	134.77	12.98	4.32
4	127			108		112				118	130			86	145.16	13.61	5.57
4½	124			108						124				84	145.00	17.56	8.78
5				108						125					157.50	16.26	11.53
									Mean starting pressure (mm Hg)						73.38	8.58	2.38

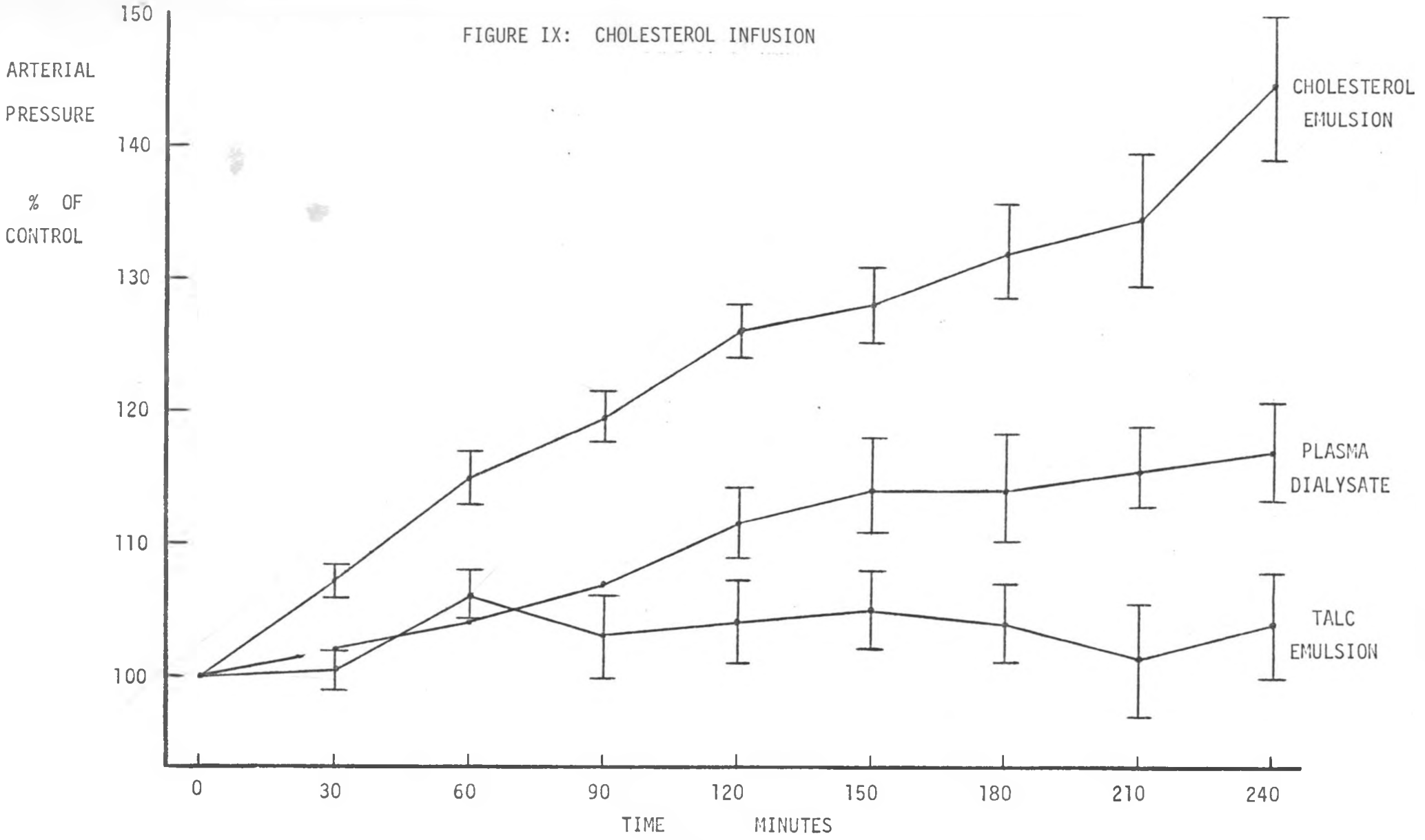
TABLE XIV: Arterial pressures at half hourly intervals in decerebrate rabbits following intravenous infusion of talc emulsion.

TIME	BLOOD PRESSURES								% OF CONTROL MEAN	S.D.	S.E.
-1	90	96	70	75	72	68	55	80			
-½	92	98	64	82	72	58	64	87	100		
0	94	94	80	88	80	64	66	85			
½	90	88	80	88	78	68	70	89	100.75	4.49	1.59
1	96	90	84	92	90	70	72	91	105.87	4.96	1.75
1½	84	92	88	92	80	64	76	86	102.37	7.65	2.71
2	86	94	90	102	86	60	80	86	105.37	10.41	3.69
2½	86	96	94	102	86	64	74	88	106.12	8.57	3.03
3	84	98	88	104	80	72	68	84	104.50	8.71	3.08
3½	80	98	90	100		60	64	84	100.71	9.72	3.68
4	86	96	90	104		64		84	103.83	9.47	3.88
4½	86	92							95.80	4.24	3.00
	Mean starting pressure (mm Hg)								81.25	11.29	4.00

TABLE XV: Arterial pressures at half hourly intervals in decerebrate rabbits following intravenous infusion of plasma dialysate.

TIME	BLOOD PRESSURES											% OF CONTROL MEAN	S.D.	S.E.
-1	86	68	76	100	80	74	60	90	60	62	72			
-½	64	70	88	88	86	84	68	82	60	64	74	100		
0	70	72	92	96	82	92	72	84	58	66	80			
½	76	76	94	100	82	90	76	80	56	64	82	101.8	4.1	1.3
1	84	84	100	100	84	92	76	76	60	60	80	104.1	8.8	2.7
1½	84			104	88	96	76	75	66	66	90	107.0	8.1	2.7
2	84			112	100	96	78	86	64	70	90	111.7	6.6	2.2
2½	90			104	96	94	85	76	72	80	94	114.3	11.3	3.8
3	90			104	100	96	86	79	60	84	96	114.2	11.7	3.9
3½	90			100	96	98	90	102	64	84	93	115.9	9.5	3.2
4	92			106	94	96	92	110	68	88	84	117.0	11.1	3.7
	Mean starting pressure (mm Hg)											78.5	12.0	3.6

FIGURE IX: CHOLESTEROL INFUSION



5 μ filter, assayed again, and five more experiments were performed using it.

Results

TABLES XIII, XIV, XV
FIGURE IX

Infusions of cholesterol emulsion at the rate of 0.3 mg/kg/hr caused a prompt rise in blood pressure which persisted throughout the four hours of infusion, and showed no sign of abating. The increase in arterial pressure at the end of four hours was 45% ± 5.5.

The infusions of talc, which were designed to serve as controls for the infusion of particles into the circulation showed a very slight increase in arterial pressure, being less than 5% ± 3.9 during the four hours of the infusion.

The infusions of dialysate of plasma containing a known amount of cholesterol at the rate of 0.3 mg/kg/hr of cholesterol showed a very slow increase in arterial pressure which did not become significantly different from the talc infusion until 3 hours, and reached a maximum increase in pressure of 16% ± 3.5. There was no difference between the effects of the plasma dialysate after only three days of dialysis and after a second three day dialysis.

The cholesterol emulsion infusions are significantly different from the two control infusions from the first 1/2 hour.

High Fat Diet

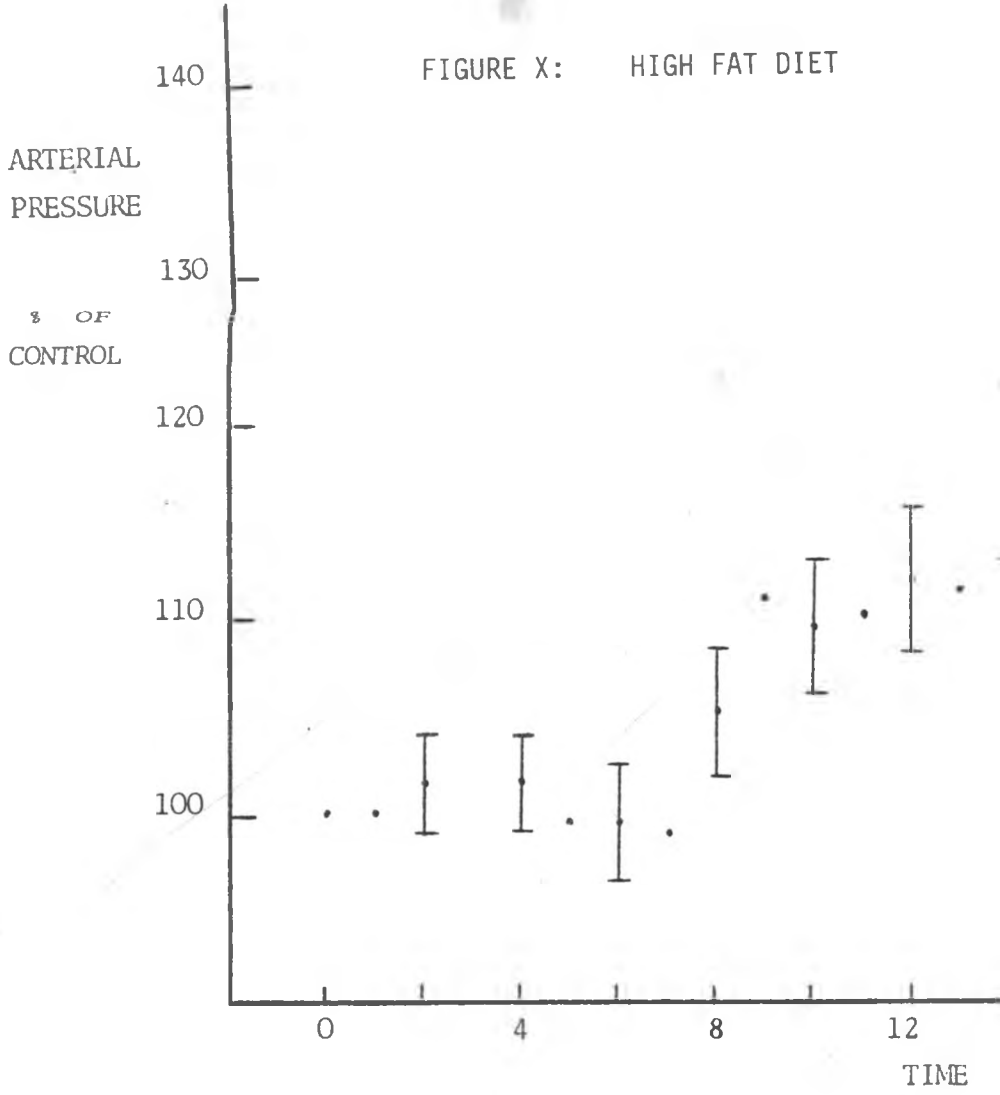
Methods

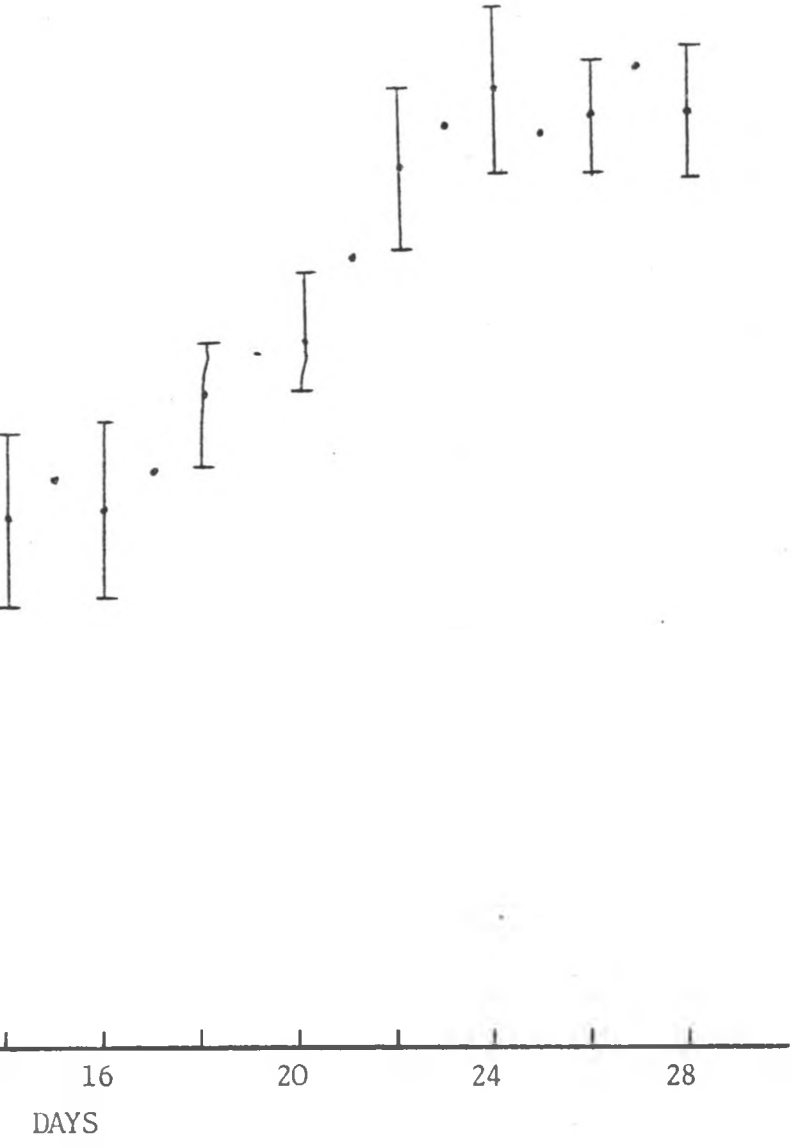
Eight rabbits were put into cages and had their arterial pressure measured daily for two weeks. These animals were maintained on a diet of rabbit pellets and water. The arterial pressure was measured by means of an ear capsule

TABLE XVI: Daily blood pressures taken from the central ear artery of rabbits before and during consumption of a high fat diet.

DAY	BLOOD PRESSURES								% OF CONTROL MEAN	S.D.	S.E.
-7	65	65	70	70	70						
-6	65	65	60	72	70	60	90	65			
-5	65	68	60	68	72	63	90	70			
-4	70	75	60	75	70	70	85	75	100		
-3	65	70	60	65	70	60	85	70			
-2	68	68	62	70	65	62	75	75			
-1	72	70	60	70	72	65	85	75			
1	65	70	58	70	68	62	90	75	100.00	4.03	1.42
2	65	70	60	75	68	65	80	82	101.50	6.88	2.43
3											
4	70	70	68	63	70	62	82	78	101.37	6.90	2.44
5	68	65	65	65	70	63	78	78	99.62	6.52	2.31
6	65	70	65		70	62	72	78	99.71	8.17	3.09
7				70		60	75	79	98.75	9.32	4.66
8	75	68	68	75	78	60	75	80	104.87	9.41	3.33
9	80	72	70	80	90	60	80	85	111.37	12.80	4.53
10	78	73	70	78	78	60	82	88	109.50	9.95	3.52
11	85	75	66	75	85	65	78	78	109.62	10.55	3.74
12	85	72	72	78	80	65	80	88	112.00	10.91	3.86
13	80	75	67		82	70	78	85	111.28	9.65	3.65
14	80	78	75		85	70	83	98	117.71	12.40	4.69
15	85	85	80		85	70	80	92	119.57	13.03	4.93
16	88	82	70		88	70	82	90	118.00	12.02	4.55
17	85	85	78		88	70	83	90	120.00	11.37	4.30
18	88	88	80		90	72	90	90	123.85	9.85	3.73
19	90	88	83		100	70	93	90	126.85	12.97	4.91
20	92	90	80		95	70	100	92	122.71	9.65	3.65
21	100	95	80		95	72	105	95	132.28	10.71	4.05
22	100	100	80		100	75	105	102	136.42	11.57	4.38
23	98	95	85		105	78	105	105	138.57	10.99	4.16
24	103	90	85		105	80	110	108	140.57	11.39	4.31
25	105	98	83		90	80	108	105	138.00	10.80	4.09
26	105	95	85		95	82	110	100	138.85	8.41	3.18
27	105	98	88			82	108	107	141.50	11.14	4.56
28	100	100	85			80	108	105	139.00	9.69	3.97
					Mean starting pressure (mm Hg)				69.5	7.17	2.54

FIGURE X: HIGH FAT DIET




16
20
24
28
DAYS

(Grant and Rothschild, 1934).

After the two week control period, during which the animals were becoming accustomed to being handled daily for arterial pressure measurement, the normal diet was changed to a high fat diet. The high fat diet was simply enough, rabbit pellets which had been soaked in warm hydrogenated vegetable fat (Kimbo) until saturated, and then cooled. Upon analysis, the high fat diet was found to contain 15% fat, as compared to 4 1/2% fat for the standard pellets. There was 0.13% cholesterol in both diets, and no free fatty acids.

The arterial pressures were taken as before during the four weeks when the high fat diet was being administered.

During the course of the experiment, blood was taken at irregular intervals and analysed for cholesterol content.

Results

TABLE XVI

FIGURE X

During the control period of arterial pressure measurement, initially, the arterial pressure was rather unstable. After one week, the animals became accustomed to daily arterial pressure measurements, and the arterial pressure stabilised. The blood pressure given for the control period only covers these last 7 days during which stability was achieved.

After the high fat diet was administered, there was no change in arterial pressure at all, until the eighth day. From this day onward, the arterial pressure climbed steadily to reach a maximum of 40% above control after twenty-four days.

During the course of the experiment, plasma cholesterol levels were assayed. Although it was expected that the plasma cholesterol would rise as a result of the fat diet, in fact, there was no consistent change whatever throughout the course of the experiment. The failure to produce an increase in

plasma cholesterol was the reason for terminating the experiment at this stage, even though it was not entirely certain that the arterial pressure had indeed stabilised.

Summary of Results

1) Intravenous infusion of aldosterone raises the arterial pressure 20% in five hours.
2) In progesterone pretreated animals, aldosterone infusion raises the arterial pressure 45% in five hours.
3) In progesterone pretreated animals, arterial pressure rises 15% during a five hour saline infusion.
4) In salt fed animals, aldosterone infusion raises the arterial pressure 30% in five hours.
5) In salt deprived animals, aldosterone infusion raises the arterial pressure 10% in five hours.
6) Interference with the ability of both carotid baroreceptors to detect elevated pressure by means of encapsulation results in a 38% chronic hypertension maintained for at least 50 days.
7) Infusions of noradrenaline are far less effective in raising the arterial pressure in decerebrate animals with lively baroreceptor reflexes than in decerebrate anaesthetised animals whose baroreceptor reflexes have been muted by the anaesthetic.
8) Aldosterone selectively perfused through the carotid sinus can raise arterial pressure. Aldosterone infused into animals whose carotid baroreceptors are externally perfused, and protected from exposure to the aldosterone, has no effect upon arterial pressure. Aldosterone infusion has no significant effect upon the arterial pressure of a nembutal anaesthetised animal.
9) Infusion of 0.3 mg/kg/hr of cholesterol emulsion in saline raises the arterial pressure 45% in four hours. Infusion of plasma bound cholesterol in the same amounts raises the arterial pressure 15% in four hours. Infusion of emulsified talc in the same amount has no effect on arterial pressure.
10) Administration of a diet high in unsaturated fats to rabbits results in an elevation of arterial pressure beginning 6 days after starting the experimental diet, and

reaching a peak of 40% above control after 24 days. During the course of the experiment, there was no change in the plasma cholesterol concentration.

CHAPTER VII: DISCUSSION

Part 1 - Chapters II, III, IV

In all of the acute experiments reported in this thesis, the rabbits were decerebrate, curarised, and unanaesthetised. This preparation maintains a stable arterial pressure from about one hour after the decerebration, until at least five hours later (Chapter II). This preparation possesses intact and lively baroreceptor reflexes while the nembutal anaesthetised animal does not (Chapter V, b).

Aldosterone infusions into decerebrate rabbits result in a rapid rise in arterial pressure (Tables I and II, Figure I). This effect is slightly but significantly enhanced by hypersalimentation and slightly, but significantly reduced by salt deprivation and greatly enhanced by pre-treatment of the animals with progesterone injections (Tables III-VII, Figures II and III).

Although the rise in arterial pressure following chronic progesterone pre-treatment (Horrobin, 1968) is prevented by simultaneous hypersalimentation (Horrobin and Lloyd, 1970), saline (control) infusions into these animals were accompanied by a significant pressure rise.

The major stumbling block in the acceptance of the role of aldosterone in the generation of the hypertension of primary aldosteronism, malignant hypertension, and some forms of essential hypertension is the evidence that aldosterone is not a potent hypertensive agent (Pickering, 1968, p. 581).

The results presented here show that aldosterone is a rapid acting hypertensive agent when administered continuously via the intravenous route. In the past (see CHAPTER II, Introduction) most of the experiments attempting to show a chronic effect of aldosterone on the arterial pressure have used intramuscular injections of the hormone. It is possible that this mode of administration is not capable of producing substantially elevated blood levels of aldosterone. This author has found (unpublished results) that intramuscular injections of aldosterone into rabbits (50 µg/kg and 100 µg/kg twice daily) were virtually

ineffective in altering the rate of sodium excretion over a period of five days. However, injections of similar amounts of aldosterone into the same rabbits intravenously produced the expected sodium retention.

It is clear that the average secretion rate/kg of aldosterone in primary aldosteronism, essential hypertension, renal artery stenosis, and malignant hypertension (CHAPTER II, Methods) are usually lower than the infusion rate/kg of aldosterone used in the experiments reported here. Nevertheless, it is proposed that elevated aldosterone secretion rate plays a part in the generation of high arterial pressure in these diseases.

Kaneko, Ikeda, Takeda, and Ueda (1967) showed that lowering arterial pressure with sodium nitroprusside in both normal people and renal hypertensives with proven renal arterial stenosis resulted in a very large increase in plasma renin activity and presumably a proportional increase in aldosterone secretion rate. The plasma renin activity in the renal hypertensive patients was five times the normal value. When their arterial pressure was lowered to normotensive levels, the already high rate of renin release from the kidney increased seven-fold. Assuming that aldosterone was responsible for at least part of the hypertension seen in these patients, then a measurement of aldosterone secretion rate with elevated arterial pressure would be misleading, because the high pressure is suppressing the aldosterone secretion rate.

In this negative feedback system, renin and the aldosterone secretion rates are the controlled variables, and the arterial pressure represents the restoring force. In a negative feedback system, there may be no correlation between the controlled variable's deviation from normalcy, and the magnitude of the system's attempt to restore normalcy. Two examples are the negligible deviation from normal of the arterial pCO_2 during exercise and the very slight temperature rise which initiates panting in dogs. The fact that a physiological quantity

deviates so little from normal a) is the best indication that it is the measured or controlled variable, b) bears no relationship whatever to the magnitude of the organism's efforts to keep it normal. Thus, for a true measure of the magnitude of the deviation of the pCO_2 from normal as a result of exercise could only be obtained if the respiratory minute volume would be kept constant. Similarly, a measurement of aldosterone secretion rate made while the patient is artificially maintained in a normotensive state would reveal the unsuppressed secretion rate, and give a better indication of the ability of the endogenous production of aldosterone to maintain the arterial pressure at hypertensive levels.

A similar argument may be made for other types of hypertension in which a secondary hyperaldosteronism is being maintained by an elevated plasma renin. The renin and aldosterone production will be partly suppressed by the hypertension, so that the relatively small quantities of these substances measurable in the blood may not appear to be sufficient to account for the degree of hypertension observed.

In the case of primary aldosteronism, this argument cannot be applied, because the steroid is produced by a tumour whose rate of secretion of the hormone does not respond to outside stimuli. The aldosterone production is associated with a low plasma renin activity and it is not responsive to either alterations in salt balance or arterial pressure, both of which are mediated by renin (Davis, 1962).

The enhancement of aldosterone hypertension by progesterone is particularly important. The two hormones tend to occur in large amounts together in pregnancy. Progesterone secretion rises throughout pregnancy (Eton and Short, 1960), and the saluresis it induces (Martin and Mills, 1956; Landau and Lugibihl, 1958) is probably the cause of the large increase in aldosterone secretion (Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook, and Greenwood, 1959; Watanabe et al., 1963). The occurrence of these two hormones together in large amounts in

pregnancy may be responsible for the occurrence of the hypertension of toxaemia. It was noted that animals receiving a threshold dose of progesterone (2.5 mg/24 hours) did not all show increase of sensitivity to the aldosterone infusions, indicating that there may be a threshold to the phenomenon. It is proposed here that hypertension in pregnancy only occurs in those women who are particularly sensitive to aldosterone hypertension, the majority of pregnancies being uncomplicated by the high levels of both steroids.

The occurrence of a rise in arterial pressure or actual hypertension in women taking oral contraceptives (Laragh, Sealey, Ledingham, and Newton, 1967; Woods, 1967; Walters and Lim, 1970; Weir et al., 1971), may also be the result of sensitisation of the individual to aldosterone by the progestin content of the contraceptive. Certainly, there have been cases of frank hypertension in women taking oral contraceptives, and these may be examples of unusually high sensitivity to aldosterone hypertension. The aldosterone secretion of women treated with oral contraceptives increased (Laragh et al., 1967) as did their plasma renin activity, and this could be due to a saluretic action of the progestins contained in the contraceptive medication similar to that reported for progesterone. It is interesting to note that the population in this part of Africa consumes very little salt in their diet by western standards, and therefore, if the hypothesis is true, the incidence of hypertension due to oral contraceptives should be higher here than in the west. A survey has not yet been undertaken to show whether this is true.

Very recent experiments from this laboratory (Horrobin, Ndeti, and Burstyn, unpublished) have shown that the hypertension caused by treatment with oral contraceptives is rapidly reduced by the ingestion of excess sodium. This result is to be expected if the saluresis resulting from progesterone and/or progestins is the root cause of the excessive production of aldosterone, which then leads to the hypertension. Similarly, the results of Robinson (1958) show that ingestion of excess

sodium during the course of pregnancy greatly reduces the incidence of pre-eclampsia. Since increased sodium intake in pregnant women is known to greatly reduce the aldosterone secretion rate (Watanabe et al., 1963), this provides indirect evidence for the importance of aldosterone in this disease.

The results of Miall (1959) bear upon the hypothesis being pursued here. He showed that there was no correlation between the salt consumption of men and their arterial pressure (in contradiction to Dahl and Love, 1954), but a negative correlation in women. Presumably, women, being sensitised to aldosterone hypertension by their progesterone secretion, will show a greater alteration in arterial pressure with alterations in aldosterone secretion rate than men. On a low sodium diet, a woman would therefore have a high aldosterone secretion rate, and a higher arterial pressure than a woman on a high sodium diet. Furthermore, it is highly interesting that the incidence of primary aldosteronism in women is three times that in men (Conn, 1963). The greater sensitivity of women to aldosterone hypertension could be due to either a sexual difference in the incidence of adrenal tumours, or to sensitisation by progesterone, the latter explanation being favoured by this author.

The result that saline infusion (control) into progesterone pre-treated animals was followed by an increase in arterial pressure was quite unexpected. This might be compared to the situation in normal pregnancy where frequently it is noted that the arterial pressure increases and remains high for some hours after delivery (Browne, 1958). This is associated with a secretion of glucocorticoids precipitated by the trauma and stress of delivery (Gemzell, 1953). In order to test this, experiments were performed using infusions of cortisol into normal rabbits and rabbits pre-treated with progesterone. Cortisol infusions caused an increase in arterial pressure and this was enhanced by progesterone pre-treatment (Muriuki, Horrobin and Burstyn, unpublished).

Hypersalimentation has been shown to increase arterial pressure in experimental animals if sufficient salt is given (Dahl, Heine and Tassinari, 1962, 1963; Dahl and Shalkow, 1964; Dahl, Knudsen, Heine, Leitl, 1968). Thus, it may be puzzling at first that hypersalimentation can relieve the hypertension due to progesterone (Horrobin and Lloyd, 1970), but if it is accepted that the hypertension is precipitated by a secondary increase in aldosterone secretion, then, excess sodium, in preventing the high aldosterone secretion, will prevent the increase in arterial pressure.

The observation that hypersalimentation enhances the hypertensive response to aldosterone infusion is more difficult to explain. Three explanations are possible. The excess sodium may have a direct effect upon the function of the baroreceptor and this is indirectly inferred by Biglieri and McIlroy (1966). It is possible that the as yet undiscovered saluretic hormone may play a part in the response to aldosterone. Last, hypersalimentation will result in a lowered secretion of aldosterone, and lowered plasma concentrations of the hormone, which itself may increase the animal's sensitivity to the actions of aldosterone. This author favours the last explanation slightly over the others, and experiments are now proceeding which may elucidate the question.

Since the progesterone pre-treated animals were also given excess sodium in their diet, it is important to separate the effects of the excess salt from those of the progesterone. It is clear that the potentiation of the hypertensive effect of aldosterone by excess salt ingestion is small, though statistically significant. Since the potentiation of the hypertensive effect of aldosterone is very substantially enhanced by progesterone pre-treatment and salt feeding, it is likely that progesterone itself would have had a significant effect, if this were not obscured by the simultaneously developing hypertension during the pre-treatment period in the absence of sodium supplements (Horrobin and Lloyd, 1970).

Part 2 - Chapter V

Interference with the ability of both carotid baroreceptors to detect raised arterial pressure (Figure IV) resulted in an immediate and sustained elevation of arterial pressure for the whole of the fifty day observation period.

Steady infusions of noradrenaline into decerebrate rabbits (Table VII, Figures V, VI) were unable to maintain the arterial pressure at a high level. The baroreceptor reflexes were tested and found to be very lively. When the same animals were then injected with an anaesthetic dose of nembutal, similar infusions of noradrenaline were able to cause very substantial rises in arterial pressure. The baroreceptor reflexes were found to be greatly muted by the anaesthetic.

Application of aldosterone to the carotid sinuses (Table IX, Figure VII) was found to raise arterial pressure. Careful cross-circulation experiments (Tables X, XI, XII, Figure VIII) restricting aldosterone only to the carotid sinuses resulted in increased arterial pressure; protecting the carotid sinuses from exposure to the exogenous aldosterone while allowing the rest of the body to come into contact with it resulted in no change in arterial pressure. These experiments suggest that one of the major sites of action for aldosterone with respect to its blood pressure raising effects is the carotid sinus baroreceptor. In these experiments it was also noted that aldosterone infusions into anaesthetised animals (Table X) resulted in no rise in arterial pressure.

The cast encapsulating the carotid baroreceptors interfered with the ability of the baroreceptor to detect elevated arterial pressure. The baroreceptor presumably continued to deliver a phasic pattern of impulses during the cardiac cycle, but the frequency of discharge (especially during systole) must have been lower. The brain would interpret the new pattern of discharge as indicating a lower than normal arterial pressure, and raise the systemic arterial pressure in an attempt to rectify the apparent problem, thereby reducing the error signal.

The systemic arterial pressure would rise until the rising discharge frequency from the remaining intact baroreceptors would cause the arterial pressure to stabilise at a new level higher than normal. This was what was observed, the arterial pressure rose within one day reaching a stable level which was maintained until sacrifice, more than fifty days later.

It has been suggested by Dickinson (1965) that brain ischemia may result following constriction of either the internal carotid or vertebral arteries via a medullary baroreceptor. This is unlikely to be involved in the hypertension caused here because of the method used to apply the cast around the carotid sinus. A clamp was put on the common carotid artery to decrease its pulsation so that a close fitting cast would result. However, the artery was still inflated, and measurements made here during the course of another series of experiments have shown that clamping the common carotid artery only decreases the pressure within the carotid sinus by about 20 mm Hg. The slightly decreased diameter of the vessel (now made permanent by the epoxy cast) would certainly have been insufficient to cause ischemia in the brain. Also, casts were applied around the common carotid arteries in two animals which then served as controls, and no hypertension developed in these.

Past experiments in which the baroreceptors were denervated often failed to produce chronic hypertension. Presumably the total lack of signals from the denervated baroreceptors was eventually interpreted as a malfunction rather than a low pressure signal by the brain which then ceased to pay attention to them, and began to use other normally functioning, but minor, baroreceptors for control. Ead, Green and Neil (1952), and Scher and Young (1963) have shown that if one merely converts the pressure in a baroreceptor from pulsatile to non-pulsatile, the brain will ignore that baroreceptor and rely on others, presumably interpreting its information as obviously false.

In the experiments reported here, the brain obviously did not start to ignore the altered baroreceptors and their

erronious discharge, because the hypertension was stable. From this it can be inferred that although the average frequency of signals sent by the altered baroreceptor must have been lower, the normal phasic pattern must have been preserved to some extent. A more important deduction to be made is that the brain continues to pay attention and rely upon the false information without "accommodating". There is considerable evidence that the baroreceptor adapts or resets in hypertension (Kezdi, 1956; Matton, 1954; McCubbin, Green, and Page, 1956; McCubbin, 1958; Alexander and DeCuir, 1966; Aars, 1968; Bristow, Honour, Pickering, Sleight, and Smyth, 1969). The experiments reported here show that the brain does not adapt to the erronious output of an altered or mechanically reset baroreceptor. An important corollary of the resetting of the baroreceptors is that any tendency to reduced arterial pressure in a hypertensive individual will be actively opposed by the reset baroreceptor, and thus, regardless of the original cause of the hypertension, the baroreceptor will work to maintain the pressure high, in the same way as it would function to maintain pressure in a normotensive individual. This is a consequence of the fact that the resetting means that the baroreceptor in the hypertensive individual will be firing at approximately the same frequency (McCubbin, Green, and Page, 1956; Aars, 1968) as in normal individuals.

The resetting of the baroreceptor is probably mainly due to the physical effects of the elevated arterial pressure (Kezdi, 1962, 1965), since protecting one carotid sinus from the elevated pressure of experimental hypertension by means of a Goldblatt clamp prevents the resetting from occurring.

The ability of the baroreceptor reflexes to maintain a steady arterial pressure was tested by infusions of noradrenaline. In decerebrate animals, the baroreceptor reflexes were found to be very lively, and infusions of noradrenaline were relatively ineffective in raising the arterial pressure despite the large amounts given. However, after the muting of these reflexes by the administration of anaesthetic amounts of nembutal, the

same rates of noradrenaline infusions into the same animals resulted in much larger increases in arterial pressure. This result indicates that the baroreceptor reflexes are very powerful and can probably deal with any reasonable perturbation of the arterial pressure. This is supported by recent experiments in which hypertensive animals and people have had carotid sinus nerve stimulators implanted for the purpose of continuous stimulation of the baroreceptor afferents. Stimulation of these afferents produced a substantial decrease in the arterial pressure, where previously drugs were ineffective in controlling the hypertension (Schwartz, and Griffith, 1965; Bilgutay, Bilgutay, and Lillehei, 1965; Tuckman, Reich, Lyon, Goodman, Mendlowitz, and Jacobson, 1967). The latter authors comment that "the baroreceptor mechanism, under maximal stimulation, can overcome hypertension produced by renal artery constriction, which presumably is on a hormonal basis, and thus effect lowering of the blood pressure to near normal levels". The potency of the baroreceptor reflex is therefore not to be underestimated.

These experiments also form the basis of the decision to use decerebrate animals rather than anaesthetised. The latter always show a diminution of the baroreceptor reflex and hence an abnormal stituation in which to test the action of a pressor substance such as aldosterone. For example, in the cross perfusion experiments where the aldosterone infusion was given to the donor animal (Table X), the anaesthetised donor showed no rise in arterial pressure whatever. These animals were not interfered with in any way apart from nembutal anaesthesia and the cannulation of both femoral arteries and veins. Their baroreceptor reflexes were operational, but testing the response to carotid occlusion revealed that they were considerably attenuated compared to the unanaesthetised recipient. This is further evidence that the site of action of aldosterone is at the baroreceptors, and that its action depends upon their normal function.

In fact, if aldosterone were acting as a peripheral vasoconstrictor, or otherwise through the vasoconstriction effectors, one would logically expect that its action would be greater in the anaesthetised animal where the opposition from the baroreceptors to a rising arterial pressure would be reduced. This is what was shown for noradrenaline infusion. However, aldosterone does not act like noradrenaline, and therefore, it is proposed that its vasoconstrictor properties are slight if any.

It must be mentioned here that Dr. Okong'o of this department has demonstrated that aldosterone has a vasoconstrictor action in ganglion blocked animals. He is at present attempting to demonstrate an effect of aldosterone on isolated arterial muscle.

The experiments of Chapter V d, show that aldosterone can act upon the carotid sinus to produce hypertension, and that protection of the carotid sinus from aldosterone will prevent its hypertensive effects. Aldosterone hypertension being mediated by the baroreceptors, may therefore be classed as a form of neurogenic hypertension.

Since the effect of aldosterone on the baroreceptor is very rapid, there are probably only two mechanisms which might be responsible for its action. It may act directly upon the receptors themselves, making them fire at a lower frequency than normal for any level of distortion to which they are subjected. Or aldosterone may affect the physical properties of the carotid sinus wall by relaxing the muscle, lowering the tension in the wall (assuming that the pertinent receptors are located in series with the smooth muscle fibers - Heymans and Neil, 1958, chapter 8), and therefore decreasing their frequency of firing. A third possibility is that the arterial wall may be stiffened, thereby reducing its compliance, which would have the effect of reducing the sensitivity of the baroreceptor.

Biglieri and McIlroy (1966) showed that the baroreceptor reflexes increase in sensitivity in patients with primary

aldosteronism after removal of the adrenal adenoma. They therefore suggest that in primary aldosteronism the baroreceptor reflexes are less sensitive than normal. They blame a deranged electrolyte balance as the cause of the resetting but their data supporting this conclusion are rather unconvincing. In any case, their experiments cast no light on the mechanism of the resetting of the baroreceptor in this form of hypertension.

In summary, it is proposed that aldosterone may cause hypertension by deranging the normal function of the arterial baroreceptors in such a way that for any given arterial pressure, the rate of generation of impulses is reduced. The brain interprets the altered input as a low arterial pressure, and takes steps to restore the baroreceptor discharge to normal, so that a neurogenic hypertension ensues. Thus, what is proposed here is a primary resetting of the baroreceptors resulting in hypertension, the resetting being accomplished by an elevated aldosterone concentration in the blood. It has been shown that the baroreceptor is able to induce the appearance of a chronic neurogenic hypertension if it is reset so as to produce a lower average discharge frequency for any given pressure. It has been shown that progesterone enhances the hypertensive action of aldosterone, and it is here proposed that this is effected by a synergistic action of these two steroids upon the baroreceptors.

The role of sodium in this scheme is not clear. It is possible that changes in plasma sodium concentration may alter the function of either the smooth muscle or the receptor via an action on the cell membrane. It is more likely that sodium loading or deprivation affect the hormone secretion pattern, and affect the baroreceptor's response to aldosterone in this way.

Part 3 - Chapter VI

Attempts to elevate the plasma cholesterol concentration by feeding a diet enriched in saturated fat failed entirely. However, the fat enriched diet resulted in a substantial elevation of the arterial pressure (Table XVI, Figure X).

The increase in arterial pressure seen during the feeding of a diet high in saturated fat to rabbits was similar to that observed by Renaud and Allard (1963). Since a high lipid diet is an essential component of most cholesterologenic diets, it is possible that the increased arterial pressure ascribed by many investigators to elevated plasma cholesterol concentrations (Chapter VI, Introduction) may have been due to some other factor resulting from the diet, rather than elevated plasma cholesterol. Certainly, in the experiments reported here (Chapter VI), the increased arterial pressure cannot have been due to elevated total plasma cholesterol, since this did not change.

Infusions of small quantities of chemically free cholesterol have a very potent hypertensive effect upon the arterial pressure, despite the fact that the total quantity infused over four hours amounted to approximately 1-2% of the total estimated blood cholesterol content (Table XIII, Figure IX). Since the infusion of the same quantity of cholesterol bound to plasma proteins had a far smaller effect upon the arterial pressure, if any (Table XV),the hypertensive effect of the unbound cholesterol must have been due to a peculiar property of chemically free cholesterol which is lost upon binding to plasma proteins. It is proposed here that the active site on the cholesterol molecule by which it is bound to its blood plasma carriers is the same site through which it exerts its action on arterial pressure. The infusion of a talc emulsion containing solid inert particles of the same size and amount as the cholesterol emulsion serves as a control (Table XIV) and shows that infusion of 5 μ or smaller solid particles could not itself be responsible for the observed rise in pressure seen in Table XIII.

The results of Kellner, Correll, and Ladd (1949, 1951) (Chapter VI, Introduction) present a similar anomaly whereby an apparently large chronic increase in plasma cholesterol concentration was associated with a lowered incidence of arterial lesions, contrary to usual experience (Mitchell and Schwartz, 1965, Chapters 13 and 14). This could be explained if, despite the increase in total assayable cholesterol, there was a decrease in the "physiologically active" chemically free form of the molecule because of binding by the intravenously injected detergents.

Similarly, Bhattathiry and Siperstein (1963), while studying the control of hepatic cholesterol synthesis, observed that though hepatic cholesterol synthesis was depressed by increased dietary cholesterol, there was no correlation between hepatic cholesterol synthesis and plasma cholesterol concentration. It is suggested that a correlation may have been found between free unbound plasma cholesterol and hepatic synthesis of the steroid, but since this cholesterol concentration is so small, a change in it would not have been detected in total assayable plasma cholesterol.

Although it is highly speculative, this author suggests that the pertinent form of cholesterol in the circulation is the very small quantity of chemically free cholesterol which can be put into watery solution. The author can conjure up no other reasonable explanation for the observation that an infusion of 1-2% additional cholesterol into the circulation (assuming approximately 200 mg% blood cholesterol in the rabbit - Biochemist's Handbook, Long, 1961) should result in a 45% rise in arterial pressure. This hypothesis can be used to explain the results of Kellner et al. (1949, 1951) which are otherwise difficult to understand. Although Bhattathiry and Siperstein (1963) did not find it peculiar that they were unable to detect a correlation between plasma cholesterol concentration and hepatic synthesis of this steroid, they nevertheless must have expected to find one, or they would not have bothered to mention their failure to do so. Once again,

the hypothesis offered here can be used to explain their findings. It has been noted previously that the correlation between plasma cholesterol concentration and arterial disease is a peculiar one (Chapter I). There is little relationship between the two variables until a plasma concentration of approximately 250 mg% is exceeded. It is therefore proposed that the free molecular cholesterol present in the circulation in very small amounts is the active form of the steroid. If this could be accurately assayed, it is suggested that a closer correlation may be found between the concentration of this chemically free cholesterol and the incidence of arterial disease, and possibly also arterial pressure.

The rapidity of action of cholesterol observed in the experiments reported here may be difficult to accept; however, a precedent exists for this. Shimamoto (1969) has shown that oral administration of cholesterol (1 gm/kg) in rabbits produces an "edematous arterial reaction" detectable within one hour which Shimamoto suggests may be the earliest stage in the generation of experimental atherosclerosis. The fact that cholesterol has a rapid action upon arterial walls is very interesting, because the author would like to speculate wildly again and suggest that cholesterol may exert its hypertensive action upon the baroreceptor in a manner similar to that already shown for aldosterone. The experiments described here, of course, provide no support for this idea.

In summary, it is proposed that there is some factor in a high lipid diet which may result in hypertension. The relevant change produced by this factor is not in total plasma cholesterol but it could be an alteration in the physicochemical form in which cholesterol is bound. It is further proposed that the chemically free form of cholesterol can cause hypertension, and it is tentatively suggested that the action of cholesterol may be exerted via the baroreceptor in a manner similar to that already proposed for aldosterone. Consequently, measurements of total plasma cholesterol may be of relatively little use in determining susceptivility to arterial disease or

hypertension compared to measurements of the minute quantity of cholesterol present in the circulation in watery solution. The ability of the blood to bind cholesterol to cell membranes and plasma proteins, or the degree of saturation of these binding sites may be a more important measurement in cardiovascular disease than total plasma cholesterol.

Part 4 - Conclusion

In the Introduction to Chapter V, the possibility that hypertension might result from a primary resetting of the baroreceptors (Volhard, 1948; Heymans, 1952) was discussed. In this thesis, it has been shown how experimental mechanical resetting of both carotid baroreceptors results in chronic hypertension. It has been implied (Chapter VI,pt 2) that since aldosterone appears to exert its hypertensive action via the baroreceptors this might also be considered a primary resetting of the baroreceptors. It has been suggested that this is the mechanism by which aldosterone initiates or contributes to raised arterial pressure in diseases such as primary aldosteronism, malignant hypertension, essential hypertension with raised aldosterone secretion rate, renal hypertension with raised aldosterone secretion rate, and hypertension of pre-eclampsia (pregnancy).

The carotid sinus area is a favourite site for the formation of fatty and fibrous placques (Bilgutay, Bilgutay, and Lillehei, 1967). The appearance of this sort of lesion in an artery containing a baroreceptor would be expected to decrease its compliance, and hence to decrease the pressure sensitivity of the baroreceptor located there. It would be expected that such a resetting of the baroreceptor would be similar to that caused by the encapsulation of the carotid artery, and therefore would be expected to raise arterial pressure.

The response of arteries to hypertension is usually to increase their elastin and collagen with a corresponding reduction in compliance (Wolinsky, 1970, 1971). It is presumably this arterial "stiffening" which accounts for the secondary resetting of the baroreceptors in some forms of hypertension (page 23). This resetting of the baroreceptors in hypertension ensures that they do not oppose the high arterial pressure, and furthermore, they will be expected to oppose any fall in arterial pressure from the hypertensive level. In experimental renovascular hypertension, the heart rate and cardiac output fall in the first few days, probably because the baroreceptors

are opposing the rising arterial pressure (Ledingham, 1971). After this initial period, both heart rate and cardiac output rise as the baroreceptors become reset. Part of the resetting is probably mechanical and follows the increased arterial pressure. It is proposed that the hypersecretion of renin and consequently aldosterone which characterises the development of experimental renovascular hypertension (Ledingham, 1971) is also partly responsible for the resetting of the baroreceptors in this type of hypertension. This resetting of the baroreceptors is presumably what delays the return of arterial pressure to normal after reversal of the renovascular hypertension (Funder, Blair-West, Cain, Catt, Coghlan, Denton, Nelson, Scoggins, and Wright, 1970). The often seen delay in restoration of normal arterial pressure following excision of the aldosterone producing tumour in primary aldosteronism may also be due to the reset baroreceptor tending to oppose the fall in arterial pressure. Progressive resetting of the baroreceptors may be a feature of malignant hypertension.

The tendency to relegate the arterial baroreceptors to a back seat when considering factors responsible for the genesis of hypertension ought to be reconsidered. It is hoped that some of the experiments described in this thesis will convince the reader of the importance of these organs in hypertension.

Further studies on the role played by cholesterol in cardiovascular disease should take into account, not only the total plasma cholesterol concentration, but the forms in which the cholesterol is to be found in the circulation, and the binding capacity of the blood for cholesterol.

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