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The effect of drugs on experimental renal hypertension in the dog.

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<https://doi.org/10.18297/etd/1852>

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UNIVERSITY OF LOUISVILLE

THE EFFECT OF DRUGS ON EXPERIMENTAL RENAL
HYPERTENSION IN THE DOG

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

Of Master of Science

Department of Physiology and Pharmacology

By

J. J. Prusmack

1937

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ACKNOWLEDGMENT

I desire to express my deepest gratitude and appreciation to Professor G. E. Wakerlin for the inspiration and guidance he has given me. I also wish to thank Dr. M. M. Weiss for his aid and interest in this investigation, and Dr. Malcolm Thompson for his assistance with the surgery on the dogs.

INTRODUCTION

Lifetimes of study have been devoted to elucidation of the intricate problems of cardiovascular physiology and pathology, and clinical applications have kept pace. However, efforts directed to the determination of the causes of so-called degenerative circulatory diseases and to the discovery of effective treatment have met failure at almost every turn. Of late years, indeed, these conditions, whether by reason of better diagnosis, greater population of susceptible age groups or other changes, appear to be increasing in frequency. A review, however of the clinical significance of changes in arterial pressure, is one of colossal magnitude from the point of view alone of the published literature referable to it, and it would seem a truism that the less a subject is understood the more is written about it.

The etiology of clinical hypertension is still largely unknown(1). Very little has been added to the knowledge of the subject since Janeway's masterly reviews in 1904(2) and 1913(3). The study of arterial hypertension really dates back to Bright(4) who was the first to observe that profound involvement of the cardio-vascular system is associated with renal disease. In his first memoir, the great clinician noted the increased resistance which the arteries of the diseased kidney offer to injection and a little later he also described the occurrence of cardiac hypertrophy in the same condition.

Shortly after Bright's communication, Toyne(5) showed that in the contracted kidney the small arteries are thickened and narrowed, thereby accounting for the difficulty that Bright had encountered in injecting such organs. In 1858, Johnson(6) found that the thickening of the small arteries in Bright's disease is not confined to the kidney, but it is also present in the arterioles of other organs.

Exact measurements of the hydrostatic pressure under which the blood exists in the large arteries and veins were first published by the Rev. Dr. Stephen Hales(7) an English clergyman, in his famous book entitled "Statical Essays, containing Haemostaticks," 1733. This observer measured the static pressure of the blood in the arteries and veins by the simplest direct method possible. After tying the femoral artery in a horse he connected it to a glass tube 9 feet in length. On opening the vessel the blood mounted in the tube to a height of 8 feet 3 inches, showing that normally, in the closed artery, the blood is under a tension sufficient to support a column of blood of this height.

Since Hales' work, the chief improvements in method which have marked and caused the development of this part of the subject have been the application of the mercury manometer by Poiseuille(8), the invention of the recording manometer and kymographion by Ludwig(9), and the later numerous improvements by many physiologists. While attempts at instrumental, indirect measurement of the arterial pressure in man date back to Vierordt(10), and extensive estimations were made by von Basch(11), Potain(12) and others, wide-spread clinical measurement of blood pressure only followed the introduction of the pneumatic cuff for compression of the artery by Riva-Rocci(13) in 1896.

Considerable differences are found in the normal arterial blood pressure of healthy individuals, the arterial tension not being held at nearly so fixed a value as is, for example, the hydrogen-ion concentration of the blood. Some of the factors associated with variations in blood pressure are known, including age, weight, sex, nutritional state, and climatic environment, as well as inborn, individual constitutional peculiarities. The range included in the normal variation of blood pressure is, however, not as broad as was formerly thought, though one is sometimes unable to say whether a

given blood pressure is abnormally high or low for the individual.

Hypertension is no more than a symptom and although the most common cause of a consistent elevation in blood pressure is essential hypertension, there are a number of other pathologic states which are regularly attended by increased arterial pressure. Essential, or non-renal hypertension in man is a constitutional, familial and hereditary disease of unknown etiology (Rohberg). There are certain rare causes of ~~a~~persistently elevated systolic and diastolic blood pressure resulting from extra-renal factors, such as suprarenal tumors, basophilic adenomas of the pituitary gland, lead poisoning, toxemia of pregnancy, increased intracranial tension, and bulbar poliomyelitis, but the vast majority of cases of persistent hypertension, not resulting from renal disease, are of unknown etiology.

The term essential hypertension must be interpreted to indicate a symptom complex rather than a disease entity, and it is probable that as medical knowledge increases, distinct types of cases will be separated from the main group on the basis of specific etiologic factors. In essential hypertension both the systolic and diastolic blood pressures are increased. Tests of renal function, in the early stages, give normal results, but as time passes, a progressive diminution in function frequently is recorded, and a certain number of patients, probably not more than 10 per cent of the entire group, ultimately die of uremia. Cardiac complications, such as congestive heart failure and coronary artery disease, are of much greater clinical importance and are the cause of death in approximately 60 per cent of all patients, while cerebral vascular accidents constitute the terminal event in 15 or 20 per cent of the cases.

Numerous attempts have been made to explain ^{the}etiology of essential hypertension. These have involved various types of experimental approach

using the human subject with essential hypertension, and also many efforts aiming at the production of experimental essential hypertension in animals. In general, these investigations have taken one of three main theses as thetiology, viz., (a) humoral, (b) nervous, and (c) renal.

I. HUMORAL

The discovery by Oliver and Schafer(14) of the profound effect of extracts of the suprarenal glands upon the blood pressure of animals, led to the hope that in this reaction was to be found the explanation for the maintenance of blood pressure, and that disturbances of this glandular function could account for abnormalities of pressure, and so, investigators have endeavored to produce a hypertension experimentally by the injection of pressor substances into animals.

A. Epinephrine. Epinephrine modifies the blood pressure by modifying the character of the peripheral circulation. When it is injected intravenously there is a prompt increase in blood pressure. This is brought about by the direct action of the drug upon the vasoconstrictor nerve endings, producing a constriction of the arterioles and thus raising the peripheral resistance. The most pronounced effect is to be seen experimentally in the vessels of the splanchnic area. On the other hand, an extract of the posterior lobe of the pituitary gland produces a rise in blood pressure, acting directly on the smooth muscle of the arterioles.

In order that the hypothesis of glandular disturbances as an etiological factor in the production of abnormal blood pressure be proven, it was deemed necessary to demonstrate the presence of epinephrine in the blood plasma, and, further, to show that this varied in amount with changes in the arterial pressure. The work of Stewart(15), Park(16), Hoskins and McClure(17)

Trandelenburg(18) and others, indicates that the amount of epinephrine in the general circulating blood is infinitesimal. On the other hand, Schur and Weisel(19) thought they had demonstrated the presence of an excess of epinephrine in the blood of hypertensive patients, because serum from such individuals, even in considerable dilution, caused dilation of the pupil of the enucleated frog's eye (Meltzer-Ehrmann reaction). However, O'Connor(20) and others have shown that this reaction is not specific for epinephrine, but it is also given off by substances arising in serum after the clotting of blood. In the plasma of the blood from the adrenal vein, there is a concentration of epinephrine from 1:1,000,000 to 1:5,000,000. In peripheral blood, either venous or arterial, O'Connor(20) found that epinephrine cannot be demonstrated. Hulse(21) workingⁱⁿ the Volhard's Clinic, found a frog perfusion preparation sensitive to a concentration of epinephrine of 1:700,000,000. He found no response of an epinephrine-like character, however, with serum or plasma of normals or essential hypertensives either with venous or arterial blood. On the other hand, if epinephrine was injected, then the serum did assume vaso-constricting properties. He also found a sensitizing substance in the blood of nephritic hypertension for epinephrine. Kure(22) et al, using very delicate chemical methods, have succeeded in detecting epinephrine in normal human arterial blood, in a concentration of 1:2,000,000. In cases of essential hypertension, concentrations of two to three times this amount were found.

Although the experimental evidence in favor of epinephrine playing an important role in the maintenance of normal vascular tone is very doubtful, yet under certain pathological conditions of the gland, such as hypernephroma, an increase in vascular tone may occur, but this is very rare. On the whole, while a few investigators still adhere to the epinephrine theory of

hypertension, it has been generally abandoned.

B. Pituitary Gland. Extracts of the posterior lobe of the pituitary body have a strong pressor action, but the experimental evidence in favor of the pituitary in the maintenance of normal blood pressure, or its effect as an etiological agent in essential hypertension is very sparse. Leimdorfer(23) injected pituitary extract intraspinally into cats, but the results were inconclusive. Menendez(24) and Menendez and Braum(25), found that dogs deprived of their hypophyses have a slightly lower blood pressure than normal, and they postulate that the lack of secretion from the anterior lobe influences the vasomotor mechanism. Cushing(26) observed a heavy infiltration of basophilic elements in the posterior lobe of fatal cases of eclampsia, and essential hypertension, and he concluded that the source of these hypertensive disorders lay in the posterior lobe of the pituitary body, that the extent of basophilic invasion from the pars intermedia was a measure of posterior lobe activity and that it represented the histopathologic basis of eclampsia and essential hypertension in young persons and may possibly be etiologically related to the atherosclerosis of old age. Kylin(27), on the basis of observations that two illnesses, Simmond's disease and essential hypertension, show opposite symptom complexes, thought that a part of the cases of essential hypertension might arise through hyperfunction of the anterior pituitary. From this observation, studies were made of the prolactin excretion in the urine of patients with hypertension, and it was shown that individuals with increased blood pressure excreted more prolactin than those with normal pressures. Blount(28) observed the influence of additional pituitary anlagen on the circulatory system of the developing urodele and found a condition paralleling hypertension in the mammal. There was marked vasoconstriction, particularly in the vessels of the gills, forelimbs and caudal fin, which may result in

stasis and obliteration of vessels. The general capillary bed was reduced, the heart rate was decreased and there was ventricular hypertrophy. These same conditions hold for hypertension in the mammal and this was the first experimental work which definitely associated the pituitary with a possible hypertensive state.

C. Pressor Action of Kidney Extracts. Almost all known urinary constituents have been considered by one investigator or another to cause hypertension as a result of retention, in the organism, the evidence for each being equally slight or entirely wanting. Urea, purine derivatives, weak acids, sodium chloride, and the hypothetical toxin of uremia have been blamed but never convicted, since upon injection into experimental animals the results have been very unconvincing, to say the least.

Tigerstedt and Bergmann(29) showed that saline extracts of the kidney tissue of rabbits produced a sustained rise in blood pressure when injected into other rabbits, and so they suggested that hypertension in renal disease was due to liberation of a pressor substance, which they named renin, as a result of destruction of renal parenchyma. Other experimental evidence along similar lines has been advanced by Hartwich(30). He found, as did Cash(31) previously, that if branches of the renal artery of a dog were ligated and the appertaining kidney tissue underwent necrosis in situ, hypertension developed, while this did not follow ligation of the splenic artery. Hartwich further found that the pressor effect of epinephrine was greater if the animal was previously given an injection of blood serum from a dog with renal tissue disintegrating in situ. On the other hand, Pearce(32) showed that the pressor action of renal extracts varied in different animals; according to him, the extract of dog's kidney produced a fall in pressure. J. L. and E. M. Miller(33) likewise did not obtain pressor effects from kidney extracts.

D. Pressor Substances in Blood. Volhard (34) included renal hypertension among the varieties of high blood pressure that he considered due to the action of a pressor substance in the blood. His pupils, Hulse and Strauss(35) claimed to have demonstrated in the blood of patients with glomerulo-nephritis and eclampsia gravidarum a substance that sensitized the arterioles to epinephrine and thereby resulted in the arteriolar spasm. They originally believed the sensitizing substance to be peptone-like; more recently, Hulse and Franks(36) have considered the possibility that it is an amino-containing body. Bohn(37) another member of the Volhard School, also claimed to have demonstrated the presence of a pressor substance in the blood of individuals with glomerulo-nephritis, the malignant phase of essential hypertension, and eclampsia gravidarum.

Petrovsky(38) reported some interesting experiments in which he found, by perfusing a normal kidney with Ringer-Locke solution, that the perfusate contained a substance which exerted a pressor action on the systemic vessels and augmented the cardiac action. He could not claim that this was specific for the kidney, as he found a somewhat similar effect produced by perfusates of other organs. The substance was thermostable and passed through a Berkefeld-Kerzen filter.

Danzer, Brody and Miles(39) obtained a marked and sustained rise in blood pressure when they injected into cats, desensitized to human blood, unchanged hypertensive blood. Agatston(40) injected fecal extract and also blood serum from patients with essential hypertension and caused spasm of the retinal artery in rabbits, but did not find this with normal serum. Weiser(41) confirmed the results of Bohn when he injected alcoholic blood extracts into cats from patients with pale hypertension and found rises in blood pressure up to 30 mm. Hg. Hantchmann, furthermore(42) observed constriction of the ear arterioles in rabbits when the ear was perfused with blood from hyper-

tensive subjects. He also found that in rabbits where hypertension was produced by tying the renal artery or by other methods, the amount of vasoconstrictor substances increased.

On the other hand, Curtis, Moncreif and Wright(42) observed no particular effect when heparinized blood from cases of essential hypertension was injected into desensitized cats. Capps and Ferris(44) failed to confirm the claim of Bohn that increased amounts of circulating substances were responsible for "pale hypertension". Leiter(45) observed pressor effects in the rat when heparinized plasma was injected, but these effects were independent of the type or even the presence of hypertension. Hypertensive plasmas did not increase the response of the blood pressure of the rat to minimal effective doses of epinephrine.

Finally, Page(46) has reported that human blood, cerebrospinal and ascitic fluid from essential hypertensives yield extracts with alcohol, which are vaso-pressor. The vaso-pressor activity was found to be dependent on the functional intactness of the central/nervous system. If the cerebro-spinal axis is severed below the midbrain, then the effectiveness of the extract is abolished in elevating the arterial pressure of anesthetized cats. Pickering(47) and others, have found that the changes in arterial pressure produced in recipients by transfusion of blood from donors with essential hypertension were very small and no greater than those produced by transfusion of an equal volume of blood from normal people.

Major(48) has advocated the view that the cause of hypertension was the retention of guanidine bases. He believed that guanidine derivatives were normal urinary constituents, and that the excretion of these substances was diminished in hypertension. Injection of guanidine derivatives into experimental animals produced a marked and sustained rise in blood pressure.

Major and Weber(86) produced a rise in blood pressure of 40-50 mm. Hg. in dogs by the injection of methyl-guanidine, and they postulate the guanidine bases as an etiological factor in hypertension.

Greenwald(49) and White(50) did not believe that there was satisfactory evidence that guanidine was excreted in normal urine.

⁽⁵¹⁾ ~~Page~~ made alcoholic extracts of the plasma of dogs with hypertension produced by the Goldblatt technique, and injected it into anesthetized cats, but obtained no greater rises in blood pressure than with extracts from normal animals. When the hypophysis was removed from such hypertensive dogs it was found that the character of the pressor response to extracts of the plasma was not altered.

(E). Cholesterol. Cholesterol is another product of metabolism which has been considered a possible etiological factor in the production of hypertension, inasmuch as Westphal(52) found that hypercholesterinemia was present in 71 per cent of cases of essential hypertension. Fishberg(1), however, stated that hypercholesterinemia was rarely striking in essential hypertension, and Buerger(53) found no relation between the cholesterol content of blood and blood pressure. Experimentally, Fahr(54) Van Leersum(55), Schmidtman(56) and Schenheimer(57) have all reported an increase of blood pressure and blood cholesterol in rabbits, occurring with arterio-sclerosis following the prolonged feeding of cholesterol in oil, or of substances high in cholesterol. There appears no doubt as to the production of arterio-sclerosis but there is a doubt as to the value of the blood pressure readings. Shapiro and Seicof(58) confirmed the production of the arterio-sclerosis but could not substantiate the increase in blood pressure. Thomas(87) described rises in blood pressure in rabbits by the repeated injection of cholesterol, but Thollidt(88) could not confirm this.

II. NERVOUS

The widespread vasoconstriction that is present in arterial hypertension is an evidence of disturbance in the normally harmonious interaction of the different vascular territories. This has been shown by the important investigations of Hering(59) and his pupils. Hering's studies were initiated by the observation that slowing of the pulse after pressure on the side of the neck was due, not to mechanical stimulation of the vague as had long been believed, but to a reflex engendered by pressure on the bifurcation of the common carotid artery. Where the common carotid divides into the external and internal carotids, there is an ampulla-like dilation which Hering has termed the carotid sinus. In the walls of the carotid sinus are nerve endings, the mechanical stimulation of which, initiates a reflex that lowers the blood pressure and slows the heart. Hering found that the sensory nerves leading from the proximal aorta--long known, especially in the rabbit, as the depressor nerve, but which he termed the aortic nerves--and the carotid sinus nerves together form a unified system that serves to prevent excessive rises in blood pressure. For this reason he termed this system of nerves the "blood pressure restrainer" (Blutdruckzugler). Hering has shown that if the blood pressure restrainers are cut in rabbits and dogs, striking arterial hypertension results. His pupils Koch and Mies(60) have maintained continuous hypertension and tachycardia in rabbits and dogs by this means for one and a half years. Radiographic and anatomical studies of the rabbits revealed dilation and then hypertrophy of the heart and there were also arterial and renal lesions. Heymans and Bouckaert(61) confirmed this work and obtained pressures up to 250 mm. Hg., by direct arterial puncture, some of their animals dying suddenly of acute pulmonary edema. Kremer, Wright and Scarff(62) observed a moderate rise in blood pressure, from 95 mm. Hg. to 115 mm. Hg., on unilateral denervation.

vation, but following bilateral denervation they found a persistent marked hypertension up to 160 mm. Hg., with degenerative changes in the aorta and fibrosis of the heart. However, Green, DeGroat and McDonald(63) noted only a transient elevation of blood pressure which at first was unstable, and then later tended to become stabilized at a level not greatly above normal. They did observe tachycardia in dogs, and sudden death from cardiac failure at times occurred, following complete denervation of the depressor system.

Pursuing a somewhat different course of investigation, De Jaegher and Van Bougaert(64) produced a generalized arterial hypertension by electrical stimulation of the floor of the third ventricle of dogs in acute experiments. Hoff and Urban(65) found that following injury to the corpora mammillaria, there was, at first, a fall in blood pressure which returned to the normal level after a few days. In one or two months a very considerable increase in blood pressure occurred, especially under the influence of excitement, and after four or five months, there was a permanent rise in blood pressure of forty to sixty mm. Hg. The objection to these methods is that they ^{appear to} have no counterpart in essential hypertension, as found in the human.

III. RENAL

The recognition of the association of renal lesions with signs of vascular hypertrophy led to a persistent idea that hypertension and nephritis were always associated. The production of hypertension through injury to the kidneys has always been considered a possibility, and as a consequence, a number of workers have attempted to answer this question by reducing the amount of functional kidney tissue in the following ways: (a) Obstruction to urinary flow; (b) Reduction of kidney substance by excision; (c) Destruction of kidney substance by X-ray; (d) Production of renal congestion; (e) Production of renal ischemia. On the whole, until lately, the results of

these various methods have been conflicting and in many instances have been open to question.

a. Obstruction to Urinary Flow. A number of observers have stated that the experimental production of urinary obstruction by ligation of the ureters was followed by hypertension and cardiac hypertrophy. This was first done by Beckmann(66) in 1857, who ligated one ureter in a dog and four months later thought he found left ventricular hypertrophy. However, Koellicker(67), the great contemporary anatomist and pathologist, examined the specimen and questioned the existence of hypertrophy. Later, Strauss(68) claimed to have produced cardiac hypertrophy in guinea-pigs by obstructing one ureter for periods of from four to six months. Much more convincing are the experiments of Rautenberg(69). He blocked one ureter in rabbits for three weeks, then removed the obstruction and extirpated the other kidney. The urine remained albuminous. He observed the animals for periods up to twenty-one months. The blood pressure rose as high as 170 mm. Hg., the normal controls having been 122 mm. Hg., or less. In dogs, Hartwich(30) has observed hypertension within three hours after the ligation of one or both ureters.

b. Reduction of Kidney Substance. Passler and Heineke(70) in 1905, first removed half of one kidney in a series of dogs. Four weeks or more later the intact kidney was removed. At a subsequent operation, another piece of the kidney operated first was resected. This was repeated one or more times at long intervals, so that as many as six operations were performed. They found a rise in blood pressure (average 21.5 mm. Hg.) by direct cannulation of the femoral artery, and cardiac hypertrophy (up to 67 per cent), that paralleled the time the animals lived in a state bordering on renal insufficiency without cachexia. With cachexia the blood pressure fell.

Bainbridge and Beddard(71) in 1907, removed three-fourths of the kidney tissue in cats and found that a loss of more than 75 per cent of the kidney

tissue caused an increase in nitrogen output, as seen in starvation, but the effect on blood pressure was questionable. Pearse,(72) in 1908, confirmed the work of Bainbridge and Beddard(71). Janeway(73) cut off part of the blood supply to the kidney of the dog by ligating some of the branches of the renal artery, and obtained a rise in blood pressure of between 40 and 50 mm. Hg. Pilcher(74) also ligated some of the branches of the renal artery of the dog, but did not obtain a rise in blood pressure or cardiac enlargement. Anderson(75) likewise, did not note any elevation of the blood pressure after removal of large portions of the kidney substance in rabbits, sufficient to produce signs of renal insufficiency. Cash(31), carried out a series of experiments on dogs in which he either separated part of the kidney from the main mass, or cut off the blood supply. He found that an increase in blood pressure, (particularly the diastolic), occurred only if he left the necrotic tissue in situ; otherwise no change was noted. This increase was maintained for a few days and then returned to normal. This view is controverted by the experiments of Paessler and Heineke(70) who did not leave any necrotic renal tissue, but nevertheless produced hypertension, and by the findings of Janeway(3) that hypertension persists long after the necrotic parts have been converted into fibrous scars. Allen, Scharf and Lundin(76) reduced the functional kidney tissue to 25 per cent of its original amount, and obtained increases of 20 to 30 mm. Hg. in blood pressure, but produced renal insufficiency. Mark(77) ligated both renal arteries and obtained a rise in blood pressure before the animal died of renal insufficiency. Mark and Geisendorfer(78) ligated one renal artery and subsequently removed the other kidney and observed a rise in blood pressure with some cardiac enlargement in their series of animals. Ferris and Hynes(79) removed one kidney, then ligated the other main renal artery

and found a rise in arterial blood pressure with a subsequent gradual fall. Apfelbach and Hensen(80) injected charcoal into the renal artery and produced renal insufficiency as a result, but no hypertension. Friedman and Wachsmuth(81) ligated the renal artery or some of its branches and found a hypertension persisting for one or two weeks. After resection of the kidney or extirpation of one kidney the blood pressure remained unchanged or fell somewhat. They did not observe a progressive rise in blood pressure or a lasting hypertension. Chanutin and Ferris(87) obtained hypertension and renal insufficiency in rats following partial nephrectomy. Wood and Ethridge(83) confirmed Chanutin and Ferris by noting chronic progressive glomerular and arterial renal lesions associated with hypertension produced by subtotal nephrectomy in rats. Pick(84) removed three-fourths of a dog's kidney tissue and observed a rise in blood pressure. When 20 cc. of the hypertensive blood was injected into a second normal dog, there was an immediate rise of blood pressure in the latter animal which stayed up for several days. Now when 20 cc of blood from a third dog, whose kidneys have been simply denervated, was injected into the second dog, its blood pressure became lowered. Other methods of reduction of kidney substance include the injection of various substances through the renal artery in an endeavor to damage the kidney to an extent just short of uremia, or to cause a blocking of the smaller vessels in the kidney. Rafsky, Bernhardt and Rhodenberg(86) injected Uranium nitrate repeatedly and produced nephritis and hypertension. They also injected cholesterol, viosterol and aspartic acid with similar results. Other workers have injected such substances as finely divided charcoal and kaolin to produce rises in blood pressure.

Bilateral nephrectomy is such a shocking and rapidly fatal operation that one would not expect any considerable hypertension during the short

tenure of life of the animals. However, Mosler(89) did succeed in keeping 13 rabbits alive for forty-eight hours after the removal of both kidneys. He observed a rise in blood pressure in all but two, but Backmann(90) was unable to confirm these results and Hartwich(30) also noted only an insignificant rise in pressure after bilateral nephrectomy in dogs.

c. Destruction of Kidney Substance by X-ray. A third way of injuring the kidney was used by Hartman, Bolliger and Doub(91). They produced in dogs extensive destruction of the renal parenchyma with replacement fibrosis and vascular sclerosis by the application of high voltage Roentgen rays to the kidneys. Consistent rise in blood pressure with hypertrophy of the left ventricle was observed in the radiated animals, which were followed for many months. Page(92) produced nephritis and hypertension in dogs by transplanting the kidneys to a subcutaneous position and irradiating with x-ray.

d. Production of Renal Congestion. Bell and Pedersen(93) produced chronic venous congestion of one kidney in the rabbit by putting an aluminum band around the renal vein and at the same time preventing development of collateral circulation with a snug membrane around the kidney. They reported a well marked hypertension of protracted duration. Menendez(94) placed a ligature on the renal vein so as to reduce its calibre without completely closing it, and produced a rise in blood pressure of over 20mm. Hg., which began two weeks after ligation and lasted more than two months. In another series of animals the kidney was denervated at the time of obstructing the vein and in none of these did he observe a rise in blood pressure.

e. Production of Renal Ischemia. All these methods of producing the arterial hypertension suffer from one of two faults; either the elevation in pressure is not persistent, or the animals die of renal insufficiency. In 1934, Goldblatt, Lynch, Hanzal and Summerville(95), in a now classic experiment, for the first time successfully produced persistent arterial hypertension

without renal insufficiency in animals. This was accomplished by establishing chronic reduction of the flow of blood to the kidneys. Eleven healthy dogs were prepared with one carotid artery looped through a short tube of skin, after the method of Van Leersum(96), to permit accurate observations of systolic blood pressure at frequent intervals; blood chemistry^{studies} and urinalyses were made to rule out the presence of renal disease. Systolic blood pressure readings were taken daily for at least two months to establish control levels; The animals were then operated on under aseptic conditions, and adjustable silver clamps were applied to the renal arteries. In some of the animals constriction was made great at the beginning, while in others it was made moderate at first and subsequently increased one or more times. Constriction of one renal artery was followed by a moderate or slight rise of pressure, which tended to return to the level of the control period. Following the production of bilateral renal ischemia, however, the systolic blood pressure rose to a variable degree in all animals. Pressures persisting between 200 and 240 mm. Hg. were common; some approached 300 mm. Hg. In two of the animals the clamping of both renal arteries was made almost complete from the beginning; the rise in blood pressure that followed was accompanied by the development of uremia, which rapidly proved fatal. In these animals the amounts of urea nitrogen, total nonprotein nitrogen and creatinine in the blood increased progressively, while the urea clearance and the output of phenolsulphonephthalein decreased progressively until death. The remaining animals survived for long periods, up to as long as three years. In only a few of these animals did tests reveal any decrease in kidney function. In one animal showing a persistent elevation of blood pressure for more than fifteen months, the urea clearance was reduced to about 50 per cent. of the mean control level. In others, however, either no change

occured in urea clearance or only slight preliminary reduction, with rapid return to normal. The concentrations of urea, total nonprotein nitrogen, creatinine and guanidine in the blood all remained within normal limits. Goldblatt and his associates, also investigated the effects of constriction of the splenic and both femoral arteries in one animal and of extirpation of one suprarenal gland with denervation and destruction of the medulla of the other in a second dog; neither of these procedures had any significant effect on blood pressure, which rose in both instances after constriction of the renal arteries. Ischemia limited to the kidneys appears to be a sufficient condition for the production of a persistently elevated systolic blood pressure. The hypertension produced by this means resembles closely that associated either with so-called benign nephrosclerosis (essential hypertension), or with so-called malignant nephrosclerosis (malignant hypertension) in man, depending on whether the constriction of the arteries is moderate or severe.

Goldblatt has also produced persistent elevation of systolic and diastolic blood pressure by renal ischemia in the macaque monkey, an animal more closely related to man than is the dog. This was a further step in the chain of analogies between the hypertension induced in dogs by renal ischemia, and that which is associated with renal arteriolar disease in man. The Goldblatt technique has afforded a new approach to the investigation of the problem of hypertension under controlled experimental conditions, and attempts to elucidate the pathogenesis of this particular type of experimental renal hypertension have followed these general lines:

- (1) Afferent impulses from nerve endings in the ischemic kidneys passing to the vasomotor center might result in reflex generalized vasoconstriction and consequent elevation of blood pressure. Complete denervation of both

kidneys before the application of the Goldblatt clamps, would give some clue as to the existence of such impulses. Page(97) unilaterally nephrectomized dogs, subsequently constricted the renal artery with the clamps and then sectioned the extrinsic renal nerves by carefully stripping the renal pedicle. He observed a sharp and sustained arterial hypertension, regardless of whether the kidneys had been denervated or not. Page and Heuer(98) in a clinical study, performed bilateral renal denervation in a patient with essential hypertension uncomplicated by detectable renal involvement, and did not find any change in the level of the arterial blood pressure. Elaut(99) repeated Page's work(97) on dogs and obtained the same results. More recently, Goldblatt, Gross and Hanzal(100) observed that in dogs, excision of the thoracic portion of the splanchnic nerves and the lower four dorsal sympathetic ganglia on both sides, does not prevent, cure nor permanently lower, in any degree, experimental renal hypertension produced by renal ischemia. These results demonstrate that the extrinsic nerve supply to the kidney does not participate in the genesis of renal hypertension, either reflexly or otherwise.

(2) Afferent impulses from the ischemic kidneys might in some way bring about increased output of some internal secretion, which, by peripheral or central action might effect general vasoconstriction and thus raise the blood pressure. Goldblatt(95) showed that the suprarenal medulla probably plays no part in the pathogenesis of this experimental hypertension in dogs. He removed one suprarenal body and destroyed the medulla of the other suprarenal after sectioning the splanchnic nerves on that side, and found that the blood pressure rise was not interfered with. Page(101) found that hypophysectomy in dogs with hypertension produced by renal ischemia (method of Goldblatt), reduced arterial blood pressure to about normal levels, and it reduced slightly the blood pressure of normal dogs. Preliminary hypophysectomy did not prevent

the rise in blood pressure established by renal ischemia, but the rise was transient.

(3) Some new substance might be transmitted humorally via the blood to effect the generalized vasoconstriction responsible for the hypertension. Renal insufficiency with accumulation of urea, creatinine and total nonprotein nitrogen in the blood, may produce an elevation of blood pressure, but the animals soon die(70). Furthermore, hypertension in dogs with renal ischemia is unaccompanied with renal insufficiency, as Goldbatt(95), Page(97) and others have repeatedly shown. Harrison, Blalock and Mason(102) prepared saline extracts from ischemic kidneys of dogs rendered hypertensive by partial obstruction of the renal arteries or by ligation of the ureters. They found that these extracts caused, first a preliminary slight decline and a secondary marked rise in blood pressure in normal, anesthetized dogs. Prinzmetal and Friedman(103) confirmed the results of Harrison et al, using similar kidney extracts from fourteen dogs with experimental renal hypertension and from fifteen hypertensive patients, whose kidneys were obtained at autopsy.

Furthermore, attempts have been made to demonstrate pressor substances in the blood of these hypertensive animals. Dicker(104) has produced a temporary rise in blood pressure in anesthetized dogs following the injection of an alcoholic extract of the serum of three hypertensive dogs, while similar extracts of normal dogs did not have this effect. Page(105) however, injected alcoholic extracts of the blood plasma of dogs with hypertension into anesthetized cats and found that there was no higher elevation in blood pressure than with extracts obtained from normal dogs. Prinzmetal and Friedman(106) perfused the tails of hypertensive dogs alternately with their own heparinized blood and with blood from normal animals and found a depressor

effect with the hypertensive blood, as compared to the normal blood. Collins and Hoffbauer(107) transfused blood from dogs with hypertension caused by the constriction of the renal arteries into normal dogs and observed no elevation of blood pressure.

In order to aid in the elucidation of the mechanism responsible for the production of experimental renal hypertension in dogs, it was considered worthwhile to study the effect of certain representative depressor and pressor drugs, acting either centrally or peripherally, in a group of animals with this form of hypertension. It was felt that a comparison of their effects in experimental renal hypertension with their already known action on the blood pressure in clinical essential hypertension and in normal animals and humans might be of value in this regard. The work reported here, therefore, was undertaken with this objective in mind.

MATERIAL AND METHODS

Dogs of mixed breeds were used. The animals, all males, varied in age and weight, although all weighed over 10 Kg. Their exact age was not known, but they were all full grown, seemingly normal dogs. They were kept in individual, roomy, sanitary steel cages and fed throughout the entire experimental period on a commercial dog food, with fresh raw meat and bones once a week. This diet was adequate to maintain the dogs in a state of good nutrition. The amount of water was not limited.

They were put thru a control period varying from one to three months during which time temperature, pulse and respiration as well as blood pressure were taken twice a week. The weight was recorded once a week and occasional blood chemistries and urinalyses were done, which invariably were found to be normal.

The temperature gave an indication of any disease process which may have been present, or was beginning to develop. If this occurred the dog was immediately isolated and discarded, if necessary. The pulse and respiration were used as an index of relaxation for the dog. Inasmuch as the blood pressure was taken on the unanesthetized dog by means of direct arterial puncture, the slight pain of the needle prick would occasionally disturb the animal during the early part of the training period, so that the pulse rate and respiration would rise due to the excitement, and higher values would be obtained for the blood pressure.

The accurate determination of blood pressure chronically and without trauma in the trained, unanesthetized animal, has occupied the attention of investigators for many years. As in humans, there are two general methods for taking blood pressure; the indirect and the direct.

The indirect method consists of a modification of the conventional sphygmomanometer as used in humans, applied to some part of the body of the animal. Van Leersum(96) made four longitudinal incisions in the skin of a rabbit's neck, and enveloped the carotid with a flap. After healing had occurred, the artery in the flap was encircled by a small sphygmomanometer cuff and blood pressure determinations were made by palpation. This method gave systolic readings only. Janeway(109) on specially selected unanesthetized dogs, applied a modified Riva-Rocci(13) cuff to the lower foreleg of large dogs, and estimated systolic pressure by palpation of the smaller arteries in the ball of the foot. Kolls(110), on anesthetized dogs, applied a sphygmomanometer, equipped with a special type of cuff which permitted compression of the artery without distortion of the leg, and a sphygmograph of sufficient delicacy to record the pulsations from an artery as small as the femoral. The systolic and diastolic pressures were estimated. Ferris and Hynes(111) used an ordinary sphygmomanometer, encircling the thigh of a dog, and a phonendoscopic type of stethoscope applied to the artery, posterior to the medial epicondyle of the femur, and determined the systolic and diastolic levels by the usual criteria. Gaertner(112) applied his tonometer to the tail of the dog, and Trendelenberg(113) used the same apparatus on the foreleg of the cat. Tonometric determinations are subject to the same objections as in man, viz., that the color changes through the skin are very difficult to distinguish and therefore, are not very reliable or accurate.

In general, there are a number of objections to the indirect method of determining blood pressure in dogs. There is the tendency for the cuff to slip or buckle up, and the difficulty of locating the small vessels of the foot and ankle and accurately applying the stethoscope to them. The method is untrustworthy because of the inability of the operator to govern the tonus of the muscles or legs since the animal "tightens up" when the cuff is inflated.

Shivering of the animal may also introduce a similar error in the readings. It is very difficult to maintain the same constancy in the selection of the correct systolic impulse below the cuff, and, at times sounds cannot be obtained. Furthermore, in attempting to determine the diastolic level the sounds sometimes fade away very gradually and may not disappear when the cuff is completely deflated. The chief advantages of the indirect, or cuff, method, are the convenience in its use and the absence of trauma.

In the direct method of blood pressure determination, the artery is either cannulated, or a needle is inserted, and connected up to a mercury or an aneroid manometer, or the recording may be made optically, (Frank(114), Wiggers(115), Hamilton(116). Pavlov(117) trained dogs to lie quietly while he inserted a cannula into a small artery on the inner aspect of the knee joint of dogs without anesthesia. Brooks(118) anchored the carotid under the skin of the neck and recorded the pressures one to three days later, by inserting a cannula or a trocar. Trendelenberg and Fleischaure(119) prepared the carotids of rabbits and made readings after recovery from anesthesia.

Dameshek and Loman(120) described an instrument for the direct determination of intra-arterial pressure in man which was considered to be suitable for the determination of the mean arterial pressure in the trained, unanesthetized dog, and this method was used in the present study and will be described later. Parkins(121) also used the Dameshek and Loman instrument and confirmed its accuracy and advantages with the trained unanesthetized dog, particularly in following the mean intra-arterial blood pressure at intervals over a long period of time.

The training of the animal was often easy, although a few dogs were so excitable that some difficulty was experienced in making them lie still. This, of course, was more often encountered in the younger animals, but, on the other

hand, these responded more readily to training. The actual training was more time consuming than any other procedure. A padded table with side supports was constructed so that the animal would lie comfortably on its back. A wide leather strap, which crossed the dog's chest and bound him down securely, was used in conjunction with two soft fabric straps, which acted to hyper-extend the hind legs. The straps were looped around the ankle and tied to the lower end of the table. A muzzle, used at first, was later discarded in favor of an ordinary collar, which was tied to the upper end of the table to prevent movement of the head. The dog was placed on the table for periods ranging from ten to thirty minutes, at about the same time each day, for a week or two. While the animal was lying still he was petted and spoken to gently, but attempts to get off the table were immediately punished. The dog was invariably fed following his release. On the second or third week, the animal was strapped down and the biweekly observations were commenced. After a rest period of a few minutes the temperature was taken rectally in the usual manner, and the pulse and respiratory rates were recorded. If the animal appeared agitated and the temperature, pulse and respiration were elevated, or any one of them was higher than ordinarily, further rest was allowed before the arterial puncture was made. This was important because the blood pressure was taken only once for that day.

The procedure of Dameshek and Loman(120) was used to obtain blood pressure readings. This consisted of a direct reading of the intra-arterial pressure by the introduction of a number twenty guage short bevel needle into the femoral artery. The needle was connected through a three-way stop-cock with a five cc. luer syringe and an aneroid sphygmomanometer. When the needle entered the femoral artery, the barrel of the syringe was pushed up rapidly and the systolic pulsations were seen. When this occurred, the valve on the

stop-cock was turned and the blood allowed to flow towards the attached sphygmomanometer. Entrance into the latter was prevented by a glass trap (originally supplied with the Becton-Dickinson spinal fluid manometer). The trap was used either empty or filled with a solution of sodium citrate. When blood entered it, systolic and diastolic pulsations became apparent and the transmitted pressure immediately began to be registered on the sphygmomanometer and the maximum pressure was obtained within a few seconds. The larger the needle used, the more rapidly was the maximum pressure obtained. With a fine hypodermic needle (guage 27), the dial moved slowly to a maximum reading, which however, was almost identical with that obtained by a large (guage 18) needle. A certain amount of fluctuation in reading was obtained, the degree of fluctuation varying directly with the size of the needle, i.e., the finer the needle, the less the fluctuation. A wait of a few seconds was made to see whether the maximum reading had been obtained, after which the valve of the stop-cock was turned to the syringe and a check was made as to the presence of the needle in the artery, and the absence of coagulation in the needle.

The blood pressure readings were made after the fluctuations of the manometer attained a more or less constant range and an average of the highest and lowest point at that instant was taken as the blood pressure. This, of course, represented neither the systolic nor the diastolic level, but a point midway, or in other words, the mean pressure was actually measured.

The actual puncture of the femoral artery in large sized dogs was not difficult, and with the development of technique, puncture of the artery was in almost all instances made at the first attempt. There was but very slight pain involved in a rapid puncture with a sharp needle and the emotional response on the part of the well trained animal was apparently extremely slight and usually absent. After completion of the readings, firm pressure was made

over the artery for several minutes which effectively prevented the formation of a hematoma. In the more hypertensive animals there was a moderate tendency for a hematoma to develop in spite of continued firm pressure.

After a control period of from two to four weeks, the dogs were subjected to a unilateral nephrectomy through a lumbar approach. The lumbar region was closely clipped and shaved from the tenth rib to the iliac crest, and from the vertebral spines to the midline. The dogs were fasted for twenty-four hours, previous to operation.

A pre-anesthetic dose of morphine sulphate 15 mgm. was given subcutaneously. Sodium pentobarbital 30 mgm. per Kg. was used intravenously for anesthesia. This combination of morphine and sodium pentobarbital gave excellent relaxation for two hours, after which the animal began to react. In three hours he was usually quite awake and was able to drink water that was offered to him.

The operative procedure for nephrectomy was carried out with strict attention to asepsis. The shaved area was washed with green soap and water, followed by ether, 70% alcohol and iodine, with a final application of 70% alcohol to remove excess iodine. After properly draping the field with sterile towels and sheets, the incision was made about two inches lateral to the costovertebral angle, along a line parallel to the vertebral column, to the third or fourth lumbar vertebra. The fascia and muscles were carefully divided and the kidney approached extraperitoneally. The peri-renal fat was then dissected away and the renal vessels and ureter identified, tied with doubled black silk, and cut. Then the kidney was shelled out and delivered through the incision. The peritoneum in that situation is very thin and delicate and tears very easily, so it was usually necessary to place several sutures in the peritoneum. Then the muscle, fascia, subcutaneous tissue and skin were sutured in layers with interrupted black silk. In several cases, infection occurred and the dogs

died of peritonitis. Usually, however, the animals made an uneventful recovery, and ate well on the second or third post-operative day.

The dogs were then carried along for a further control period following the nephrectomy, during which time the blood pressure, pulse, respiration, temperature, weight and general appearance were recorded. At intervals blood chemistry studies and urinalyses were made and eye grounds were studied, but renal function tests were not done.

Thirty to one hundred and twenty days later, the animals were subjected to constriction of the artery of the remaining kidney. The preparation, anesthesia and surgical approach were identical to that employed for the nephrectomy. The renal artery was easily identified by palpation and it was isolated for about two centimeters, near its origin from the aorta, for a distance sufficient to permit the application of the clamp. A complete and detailed description of the clamp and the instruments necessary for its application, will be found in Goldblatt's publication(95).

The amount of constriction of the renal artery could not be exactly determined, but the constriction was carried to a point where a definite palpable thrill was felt just distal to the clamp. This ensured renal circulation so that the animals did not develop uremia, yet there was sufficient constriction of the artery to cause renal ischemia and the development of a persistent hypertension.

When the hypertension had reached a fairly constant level, which occurred in four to five weeks, the effects of the intravenous injections of various drugs were observed. The drugs investigated were amyl nitrite, nitroglycerine, sodium nitrite, ether, sodium pentobarbital, histamine, phosphate, acetylcholine bromide, pituitrin, and epinephrine.

The administration of the drugs was relatively easy. Amyl nitrite was given by inhalation by means of an ether cone. The drug was obtained in the usual form of "perles" (Lilly), each capsule containing five minims. The perle was placed in a gauze sponge and crushed just previous to its administration. Nitroglycerine, 0.1 mgm. per Kg., was injected intravenously into saphenous vein on one side or the other. The same route was employed for sodium nitrite, 5 mgm. per Kg.; sodium pentobarbital, 25 mgm. per Kg.; acetylcholine bromide, 0.05 mgm. per Kg.; ^{histamine phosphate 0.05 mgm. per Kg.;} Pituitrin (P.D. & Co.), 10 pressor units per cc., and Adrenalin HCl (P. D. & Co.), 0.01 ^{mgm.} per Kg. Ether was administered to these animals by the drop method to the point of surgical anesthesia, and maintained there during the reading of the blood pressures.

The blood pressure was taken just previous to the intravenous injection of the drug, and the needle was permitted to remain in the artery during the entire response. At intervals, the needle was cleaned out with a stylet when a clot formed, but the needle remained clear in most cases for about ten minutes. This procedure worked very satisfactorily for the observation of the action of all these drugs.

Fig 1. Dog 1-2 Blood Pressure Following Left Nephrectomy and Subsequent Constriction of Right Renal Artery.

Fig. 2. Dog 2-6. Blood Pressure Following Left Nephrectomy and Subsequent Constriction of Right Renal Artery.

RESULTS

The blood pressure of these animals showed the following course. In animal 1-2 (Fig. 1), the average mean blood pressure in the control period preceding nephrectomy was 95 mm. Hg. Immediately following left unilateral nephrectomy, there was a sharp drop which lasted for several weeks, with a subsequent gradual and sustained rise, concomitant with an increase in weight and an improvement ^{of the animal's} general condition. An interval of five months was allowed to intervene between nephrectomy and constriction of the other renal artery, and during this time the average blood pressure was about 111 mm. Hg. Constriction of the right renal artery was done by turning the Goldblatt clamp 315 degrees, so as to partially occlude the lumen. This induced a rapid but moderate rise in blood pressure from a pre-operative level of 112 mm. Hg. to 130 mm. Hg. post-operatively, within twelve hours. Following this there was a more marked rise to 170 mm. Hg. within two or three weeks. The pressure level dropped approximately to 150 mm. Hg. during the following month.

Animal 2-6 (Fig. 2), was nephrectomized after a control period of four weeks, during which time the mean blood pressure was about 120 mm. Hg. After a second control period of 16 weeks with an average mean blood pressure of 125 mm. Hg., constriction of the left renal artery was done by turning the clamp 270 degrees from the open position. There was a moderate, but immediate, rise of 22 mm. Hg. in sixteen hours, and of 78 mm. Hg. within one week. This rise has persisted for three weeks at an average value of 175 mm. Hg., but there have been rather wide fluctuations around this level.

Dog 3-1 (Fig. 3), has had a somewhat shorter control period, but his blood pressure variations have been very similar to those described above.

Fig 3 Dog 3-1 Blood Pressure Following Right Nephrectomy and Subsequent Constriction of Left Renal Artery.

Fig 4. Dog 4-1 Blood Pressure Following Right Nephrectomy and Subsequent Constriction of Left Renal Artery.

Since in the other two animals it was shown that nephrectomy produced very little, if any effect on the blood pressure, it was decided to shorten the control period previous to nephrectomy and utilize the period subsequently as part of the control interval previous to constriction of the renal artery. During the control period, the mean average blood pressure in this dog was 107 mm. Hg., with fluctuations of 20 mm. Hg. Three months after the beginning of the control period, the left renal artery was constricted by turning the Goldblatt clamp 360 degrees. The blood pressure rose immediately, and in four days attained a peak of 154 mm. Hg. Following this, the blood pressure has fluctuated, but it has remained above the highest level reached during the control period.

Dog 4-1 (Fig. 4), has been subjected to the same procedure with similar results to those in the animals previously mentioned. The preliminary control period was also shortened and combined with the post-nephrectomy period. The average blood pressure for this interval was about 95 mm. Hg., with minimum and maximum readings of 88 and 108 mm. Hg. respectively. Constriction of the left renal artery was performed eight weeks after the commencement of the control period, and the clamp was tightened by two complete turns. The blood pressure soon rose and reached a peak of 138 mm. Hg. on the second day. There was a brief drop on the tenth post-operative day, and a secondary rise occurred which caused the pressure to go up to 150 mm. Hg., where it has remained for the past six weeks.

The results with the drugs studied were as follows:

1. Amyl Nitrite. Amyl nitrite elevated the blood pressure in all these animals. In dog 1-2 (Fig. 5), there was a brief rise of 25 mm. Hg. lasting for two minutes and then a subsequent fall in blood pressure to somewhat below the original level for seven minutes. The blood pressure of dog

Fig 5. The Effect of Amyl Nitrite on the Blood Pressure of Four Hypertensive Dogs.

↑↑ Amyl Nitrite 0.3 cc by Inhalation

Dog 1-2

" 2-6 _____

" 3-1

" 4-1 -|-|-|-|-|-|-|-|-|-

Fig. 6. The Effect of Nitroglycerine on the Blood Pressure of Four Hypertensive Dogs.

↑ Nitroglycerine 0.1 mgm per Kg Intravenously

Dog 1-2 _____

" 2-6 - - - - -

" 3-1 _____

.. H-1 -{--|--|---|---|---|---|---|---

2-6 (Fig. 5) was somewhat more irregular. After a transitory rise of 15 mm. Hg., there was a slight temporary drop in blood pressure, which then returned to a level slightly higher than at first. Animal 3-1 (Fig. 5), showed a marked pressor action with amyl nitrite. The blood pressure rose from 150 mm. Hg. to 190 mm. Hg., but the elevation lasted only five minutes. In animal 4-1 (Fig. 5), there was a rise in blood pressure from 150 to 205 mm. Hg. within two minutes and it did not come back to its original level for eight minutes.

2. Nitroglycerine. When 0.1 mgm. per Kg. of nitroglycerine was injected intravenously in dog 1-2 (Fig. 6) the blood pressure dropped from 138 to 50 mm. Hg. within four minutes or less, and then there was a progressive, slow rise which came back to a level slightly above the original level in ten minutes. The blood pressure fall in animal 2-6 (Fig. 6) was greater and more rapid. It went from 155 to 20 mm. Hg. in three minutes, and then quickly returned to 120 mm. Hg. in three more minutes. From that point it gradually proceeded up to its original level and surpassed it at the twentieth minute, after which it remained at a definitely higher point. The recorded blood pressure of animal 3-1 (Fig. 6) dropped to practically a zero level momentarily, and then fluctuated around the 10 mm. mark for several minutes. During this time the animal manifested convulsions and urinary and fecal incontinence, but he soon recovered when the blood pressure went up to higher levels. On the seventeenth minute the pre-injection point was reached. Dog 4-1 (Fig. 6) also showed a rapid fall from 138 to 50 mm. Hg., but the subsequent rise was more gradual. The period of recovery extended over thirteen minutes and was followed, here again, by a slightly elevated plateau which persisted during the succeeding four minutes of observation.

Fig 8. The Effect of Ether Anesthesia on the Blood Pressure of Hypertensive Dogs.

↑ Induction of Ether Anesthesia

Dog	1-2	-----
"	2-6	-----
"	3-1	-----
"	4-1	- - - - - - - - -

Fig. 9. The Effect of Sodium Pentobarbital on the Blood Pressure of Four Hypertensive Dogs.

↑↑ Sodium Pentobarbital 25 mgm per Kg I.V.

Dog 1-2 —————
 " 2-2
 " 3-1 - - - - -
 " 4-1 -|-|-|-|-|-|-|-|-|-

Fig. 10. The Effect of Histamine Phosphate on the Blood Pressures of Four Hypertensive Dogs.

↑↑ Histamine Phosphate 0.05 mgm. per Kg. I.V.

Dog 1-2 _____

" 2.6 _____

" 3-1 _ _ _ _ _

" 4-1 -|-|-|-|-|-|-|-|-|-

3. Sodium Nitrite. Sodium nitrite, 5mgm per Kg. intravenously during the period of observation, in Animal 1-2 (Fig. 7) showed a gradual decline in blood pressure from 160 to 100 mm. Hg. over a period of six minutes, but soon it rose to its previous level in four minutes. Dog 2-6 (Fig. 7) showed a slow fall in blood pressure from 190 to 120 mm. Hg., where it remained for three minutes, and then went up to 190 mm. Hg. during the ensuing four minutes. In dog 3-1 (Fig. 7) the fall was from 135 to 80 mm. Hg., the blood pressure remained at this latter mark for two minutes, and then rose to the pre-injection level. Animal 4-1 (Fig. 7) showed a gradual and moderate fall in blood pressure from 150 to 90 mm. Hg. in four minutes with a subsequent rise to normal, similar to that shown by dog 3-1 (Fig. 7).

4. Ether. Ether, by inhalation in an anesthetic dose, elevated the blood pressure of animal 1-2 (Fig. 8) from 142 to 160 mm. Hg. Dog 2-6 (Fig. 8) showed much the same thing. Its rise was from 190 to 200 mm. Hg. In animal 3-1 (Fig. 8) the rise was from 150 to 160 mm. Hg. Dog 4-1 (Fig. 8) also showed a small rise from 140 to 148 mm. Hg.

5. Sodium Pentobarbital. Sodium pentobarbital, 25 mgm. per Kg., intravenously in dog 1-2 (Fig. 9) produced a slight fall in blood pressure from 162 to 150 mm. Hg. In dog 2-6 (Fig. 9) the blood pressure was depressed from 190 to 170 mm. Hg. Dog 3-1 (Fig. 9) showed a slight fall from 145 to 130 mm. Hg., and in dog 4-1 (Fig. 9) it went from 150 to 140 mm. Hg., but varied between those two levels subsequently.

6. Histamine Phosphate. Histamine phosphate, 0.05 mgm. Kg. intravenously, produced a marked and rapid fall in blood pressure, (Fig. 10) which began in about one or two minutes following injection, and lasted four or five minutes. This was followed by a moderate rise, which soon subsided, and the final blood pressure remained slightly above the original level during the period of observation. The greatest drop was 125 mm. Hg. in one of the

Fig. 11. Effect of Acetylcholine Bromide on the
Blood Pressures of Four Hypertensive Dogs.

↑ ↑ Acetylcholine Bromide 0.05 mgm per Kg. I.V.

Dog 1-2 —————
 " 2-6 - - - - -
 " 3-1 - · - · - · - · - · - · - · - ·
 " 4-1 · · · · ·

Fig. 13. The Effect of Epinephrine Hydrochloride on the Blood Pressures of Four Hypertensive Dogs.

↑↑ Epinephrine HCl 0.01 mgm per Kg. Intravenously

Dog 1-2 —————

" 2-6 - - - - -

" 3-1

" 4-1 -|-|-|-|-|-|-|-

less hypertensive animals, 3-1, and the smallest depression of 40 mm. Hg. was in the most hypertensive dog, 2-6. Also, in this animal, the subsequent rise following the depression was higher and more prolonged.

7. Acetylcholine Bromide. Acetylcholine bromide, 0.05 mgm per Kg., when injected intravenously, produced an immediate but transient lowering of blood pressure (Fig. 11), with a somewhat more gradual recovery than with histamine. There was a fleeting compensatory elevation in two dogs, and in the other two dogs there was a slight continued depression of the blood pressure during the period of observation, which was more pronounced in the more hypertensive animal, (Dog 2-6).

8. Pituitrin (P.D. & Co.) When ten pressor units of U.S.P. pituitrin was injected intravenously (Fig. 12), it produced a very moderate rise in blood pressure, which was sustained for a fairly long period of time. The rise ranged from 12 to 30 mm. Hg., and was least in animal 2-6, (Fig. 12). The duration of the elevation of blood pressure varied from ten to seventeen minutes, and was shortest in 2-6, the most hypertensive animal. However, the highest and most prolonged rise did not occur in the least hypertensive dog, but in one of the animals with a moderate elevation of blood pressure, (dog 4-1, Fig. 12).

9. Epinephrine Hydrochloride. The effect of the intravenous injection of 0.01 mgm. per Kg. of epinephrine hydrochloride was to elevate the blood pressure almost immediately to a very high peak, where it remained only momentarily and soon fell back to its original level. The height of the rise was from 230 mm. Hg. in dog 3-1 (Fig. 13) to 300 mm. Hg. in dog 2-6 (Fig 13). The duration of the elevation in blood pressure was from four minutes in dog 2-6 to seven minutes in dog 4-1 (Fig. 13).

DISCUSSION

The Goldblatt method of producing hypertension by renal ischemia in dogs has been found to be dependable, easily reproduced, and amenable to many lines of investigation as to the possible pathogenesis of essential hypertension in humans. In the work presented here, the method has been modified slightly by first unilaterally nephrectomizing the experimental animal and then clamping the other renal artery. In all the animals there was an immediate and marked rise in blood pressure, which was more pronounced in two of the dogs. No doubt if the clamps were tightened down more on the two remaining animals, the blood pressure there would also be at a higher level. These elevations of blood pressure have persisted for six weeks to three months in the animals, and are much above the blood pressures reached at any time during the control period.

The average mean blood pressure in all these animals was about 110 mm. Hg., with extremes of from 84 to 130 mm. Hg. during the control period. These figures are in close agreement with Parkins⁽¹²⁾ values of 107.2 mm. Hg. for the average blood pressure in 35 trained, unanesthetized normal dogs, with extremes of from 92 to 120 mm. Hg. He also used the Dameshek-Loman instrument and obtained the blood pressure by direct intra-arterial puncture.

As has already been stated, the etiology of essential hypertension is still unknown.⁽²³⁾ However, the method of producing experimental hypertension by the Goldblatt technique, appears to have opened a new avenue of approach to this problem. Thus there are certain aspects of the experimental renal hypertension produced in dogs by renal ischemia, that are strikingly similar to the essential hypertension of humans. For example, with moderate constriction of the renal artery, the blood pressure becomes elevated, but there is no sign of reduced renal function. In this respect the hypertension in these animals is similar to benign nephrosclerosis or essential hypertension in man.

Almost complete constriction of the artery in Goldblatt's experiments resulted in great elevation of blood pressure which was accompanied by severe disturbance of renal function and uremia. This resembles the type of hypertension which is associated with malignant nephrosclerosis or malignant hypertension in humans. In view of these suggestive findings it seems of interest to present further experimental evidence for or against additional analogies between the Goldblatt type of hypertension and essential hypertension as it exists in humans. Hence this study was undertaken to determine the effect of certain drugs, the actions of which in the normal dog, and in normal and hypertensive humans are well known, on the Goldblatt type of experimental hypertension.

The vasodilatory properties of the nitrite group have been known for a long time, and recently it has even been claimed that minute amounts of nitrites are normal constituents of the human blood (125). The nitrites act chiefly, if not entirely on the peripheral vascular system and produce vasodilation, probably as a result of direct action on the smooth muscle of the vessel walls. The site of their activity is chiefly the arterial side of the vascular bed. In the four dogs used here, it was found that five minims of amyl nitrite by inhalation elevated the blood pressure, rather than lowered it. This was probably due to the fact that the drug was administered in the usual way by inhalation, but the vapor proved irritating to the animals and they responded by struggling. This caused an elevation in blood pressure so marked that it overcame the transitory depressor effect of the amyl nitrite. Furthermore, the amount of amyl nitrite actually inhaled in some of the cases, was so small that it probably did not produce much of a vasodilator effect in the first place. As is constantly observed in the normal animals ^{and} studied, in humans, both normal and hypertensive, nitro-

glycerine had a more profound but shorter depressor action in the hypertensive animals than did sodium nitrite.

Histamine phosphate acted as a potent vasodilator in all four of the animals, but the drop in blood pressure here again was transient. These results, of course, are in agreement with the well demonstrated depressor action of histamine in the normal dog and human. Weiss(124) however, reported that in normal human subjects, given an intravenous injection of histamine in doses of 0.001 mgm per Kg., there was no appreciable lowering of the arterial blood pressure. Moreover, he found the same thing true in hypertensive humans. The difference between Weiss' results and our own is undoubtedly due to the fact that the dose which he employed was only 1/500th of the one used by us.

The work of Dale and his associates(125) has amply demonstrated that choline, a derivative of lecithin, and its esters, represent potent vasodilator constituents of tissue extracts, and it is a remarkable fact that the introduction of an acetyl radical increases the physiologic potency of choline in reducing the arterial pressure by one hundred thousand times. In the circulation, however, the effect of acetylcholine is only transient, owing to the fact that it is promptly destroyed in the blood stream. This was found to be the case in all the experimental animals that were subjected to the intravenous injection of acetyl-choline. Our results demonstrate that acetylcholine has a depressor effect in dogs with experimental hypertension similar to its depressor action in normal dogs. However, Weiss(124), again finds that even when as large a dose of 1 Gram of acetylcholine is infused intravenously in the normal and hypertensive human over a period of ten minutes, the arterial pressure either remains normal, or is only slightly reduced. Here again, however, the dose employed by Weiss is approximately 1/25th of that used by us, which very likely accounts for our positive, and his negative, findings.

Ether by inhalation in normal dogs is well known to cause a slight rise in the arterial blood pressure(127). Collins and Hoffbauer(107) found that morphine-ether anesthesia does not lower the blood pressure of dogs with experimental renal hypertension, but has a tendency to elevate it slightly. Our findings for ether are in agreement with those of Collins and Hoffbauer, although morphine was not employed in our series of animals.

Sodium pentobarbital in anesthetic doses intravenously, caused a slight lowering of the blood pressure, which did not exceed 20 to 25 mm. Hg. in the most hypertensive animal. In general, the barbiturates in anesthetic dosages in normal dogs produces no appreciable lowering of the arterial blood pressure(128). Walter and Pijoan(129), found that sodium pentobarbital anesthesia caused but a slight fall in blood pressure from 200 to 190 mm. Hg. systolic, in a dog with hypertension due to renal ischemia, (method of Boldblatt). This is about the degree of fall observed in the dogs investigated here. In no case did the blood pressure fall to normal levels.

The response to the pressor drugs, pituitrin, and epinephrine, is similar to that obtained in normal dogs and humans. The pressor response to pituitrin is slower and its action is more prolonged than with epinephrine, and the total height of the blood pressure rise with the former drug does not exceed 40 mm. Hg., in one of the moderately hypertensive animals. On the other hand, the most hypertensive dog had the least rise, which was 20 mm. Hg. This difference probably resulted from the fact that the arteriolar musculature, already contracted down to a greater extent in the latter dog, did not respond as much to the direct stimulating action of pituitrin, as did the less hypertensive dogs. Epinephrine produced the sudden, rapid and transient rise in blood pressure so characteristic of its usual action in the normal dog. Reflex vagal slowing of the heart was also in evidence at the height of the pressor response. To our knowledge, no studies have been made of the action

of these drugs in the human with essential hypertension.

The results of these experiments, therefore, indicate that the nitrites, histamine, acetylcholine, ether, sodium pentobarbital, pituitrin and epinephrine, produce the same qualitative changes in the arterial blood pressure in dogs with experimental hypertension due to renal ischemia, as in normal dogs, and in normal, and essential hypertensive humans, to the extent that data for comparison are available. So far as the action of these drugs is concerned, therefore, the hypertension present in Goldblatt dogs resembles that of the human with benign nephrosclerosis.

SUMMARY

1. Persistent hypertension was produced experimentally in four dogs by means of the Goldblatt technique of renal ischemia, following unilateral nephrectomy.

2. Transient lowering of the blood pressure in these hypertensive dogs, was produced by the nitrites, histamine and acetylcholine.

3. Ether anesthesia elevated the blood pressure slightly.

4. Sodium pentobarbital slightly reduced the blood pressure.

5. Pituitrin and epinephrine, produced a more or less characteristic temporary rise in blood pressure in these animals.

6. The drugs employed in this study, therefore, insofar as data for comparison are available, produced blood pressure effects in these four hypertensive dogs, which are analogous to those observed in the normal dog, in the normal human, and in the human with essential hypertension.

7. These findings constitute additional evidence of a similarity between the experimental hypertension due to renal ischemia in dogs and essential hypertension in man.

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