VOLUME II
Review of Experimental Methods of Producing Vascular Hypertension.

The absence of evidence in favour of a "pressor body" origin of renal hypertension led to renewed interest in the possibility of a nervous link between the kidneys and the central nervous system being of importance in this connection. This possibility was suggested as long ago as 1905 by Loeb who, however, advanced no concrete evidence in its support. More recently, Menendez (1933) repeated the hypothesis and reported a small series of experiments in its support. He produced hypertension in dogs by constricting the renal vein. He found that the hypertension did not occur if the kidneys had previously been denervated. Having mastered a reliable technique for measuring blood pressure in an experimental animal it was, therefore, decided to select a reliable method of producing renal hypertension and then to study the effects of renal denervation on such a hypertension. Prior to reporting the results on this research there is given a review of the various methods that have been employed in an attempt to produce hypertension followed by an account of current views on renal innervation.

There/
There have been three lines of approach to the problem of chronic arterial hypertension in animals,-
(1) by interference with the kidneys; (2) by denervation of the carotid sinuses and section of the aortic depressor nerves or the "Hochdruckzügler" of Koch and Mies; (3) by the production of chronic elevation of intracranial pressure.

The methods that have been employed to damage the kidneys are, - (1) direct operative interference; (2) irradiation of the kidneys by X-rays; and (3) the injection of nephrotoxic substances.

Operative Interference: Mosler (1912) carried out bilateral nephrectomy in rabbits and observed the blood pressure just before the operation, and 48 hours afterwards, by means of a cannula in the carotid artery. In 11 out of 13 animals there was an elevation of pressure of up to 25 mm. Hg., the average elevation being plus 12 mm. Hg. His observations are open to the possible criticism that at the final observation of pressure the only functioning carotid artery was cannulated, thus lowering the pressure in the carotid sinus which would in itself induce just such a rise of pressure as he mentioned. He suggested that the hypertension was/
was due to a retained pressor body. Furthermore, he was perhaps rather premature in his final recording of pressure as from personal observations the average period of survival after bilateral nephrectomy in the rabbit is over 100 hours.

Paessler and Heineke (1905), using dogs, removed half of one kidney. Four or more weeks later they removed the intact kidney, and they then resected a portion of the remaining half kidney. This procedure was repeated one or more times at long intervals, until in some animals as many as six of such resections were performed. They studied the blood pressure by means of a cannula in the femoral artery. These operations were followed by a rise of pressure averaging 21.5 mm. Hg, which persisted long after any necrotic renal tissue must have been converted to a fibrous scar. They further observed left ventricular hypertrophy of up to 17 per cent, calculated on the basis of the ratio of the weight of the left ventricle to the weight of the right ventricle. They further noted albuminuria and casts. Blood pressure observations were of necessity very few.

Janeway (1909) carried out a similar research
in which the pressure was recorded by a modified Riva-Rocci cuff round the foreleg of the dog, the point of disappearance of blood flow being observed by palpation of the dorsalis pedis artery. He states that this latter procedure was difficult and indeed was only possible in selected animals. He reduced the functioning renal tissue by ligating branches of the renal artery. He found in all animals a rise of pressure which was sustained except where the cachexia of renal failure supervened. It appears that the hypertension persisted long after the complete fibrosis of the infarcts.

Allan, Scharf and Lundin (1925) carried out partial nephrectomy in dogs, sheep and goats. They found that dogs will die if more than 75 per cent of the kidney tissue is removed, while goats will survive in apparently good health with only 1/5th of one kidney. In dogs on a normal diet elevations in blood pressure from 20 - 30 mm. were obtained. Mark (1928) produced a notable renal insufficiency in 5 dogs by means of ligation of branches of the renal arteries. These animals continued in good health. His observations on these animals on a control diet were as follows: - There was no polyuria/
polyuria. There was a definite loss of ability to concentrate urine but no loss of ability to dilute it. There was a loss of ability to excrete definite amounts of ingested urea as compared with normal animals. No hypertension was observed.

Allan, Ballman and Mann (1935) have published an account of the effect of resection of large fractions of renal substance on dogs. Estimations of blood pressure made by an indirect method (nature unstated) and also by the direct method did not reveal hypertension.

Anderson (1926) using rabbits, removed a portion of one kidney and 10 days later he removed the other kidney, thus depriving the animal of 60 - 70 per cent of its renal tissue. The blood pressure was observed by his own apparatus, employing the central artery of the ear. He observed no hypertension. There was, however, definite increase of blood urea and creatinine. Histologically, he noted hypertrophy of the renal remnant which was more pronounced when the animals were fed on a high protein diet.

Mark and Geisendorfer (1930) produced renal insufficiency in dogs by ligation of the renal arteries.
arteries and subsequent removal of the remaining kidney. They demonstrated a definite hypertension and cardiac hypertrophy in those animals that survived the operation for an appreciable length of time. Hartwich (1930), using dogs and observing blood pressure by means of a femoral cannula, found that neither unilateral or bilateral nephrectomy was associated with hypertension. On the other hand he found that unilateral or bilateral ligation of the ureters, or the unilateral ligation of a main branch of the renal artery, resulted in hypertension.

Ferris and Hynes (1931) removed one kidney and ligated the renal arteries of the other. Pronounced rise in blood pressure occurred shortly after the operation. This elevation was followed by a gradual fall to a level some 10 - 15 mm. above that of the control period. Apfelbach and Jensen (1931) injected particles of charcoal into the renal artery. They noted pronounced nitrogen retention with evidence of acidosis, but there was no evidence of arterial hypertension.

Chanutin, Ferris and Barkdale (1932, 1933) have made a detailed study of the effect on rats of the/
the progressive operative reduction of functioning renal tissue, combined with functional overstrain produced by high protein diets. In general, their results showed that in partially nephrectomised animals the degree of renal insufficiency, albuminuria, retention of nitrogen and, to a certain extent, the occurrence and degree of hypertension were found to be more pronounced when the animals were on diets rich in protein. One feature of the work of Chanutin et alia is the careful correlation between pressure and cardiac hypertrophy.

Cash (1924) reported a series of ten experiments in which he used dogs and observed the blood pressure by Koll's modification of the Erlanger method consisting of a Riva-Rocci cuff round the animal's hind leg and attached to a manometer recording on a smoked drum. The pressure is reduced in the system and the systolic pressure is taken as the point at which excursions of a definite amplitude appear; the diastolic pressure is taken as the point at which the maximal excursions begin to show slight reduction. He concluded (1) that reduction of renal tissue by excision and ligation of renal vessels was followed under/
under certain conditions by a rise both in systolic and diastolic pressure; (2) The conditions under which this change occurred were (a) the reduction of total amount of renal tissue by at least 50 per cent, and (b) the remaining in situ of a portion of kidney which has been deprived of its circulation; (3) Extensive necrosis of renal tissue alone, such as could be produced by widespread bland infarction of one kidney, or the ligation of one branch of the renal artery, was not sufficient to cause any rise of blood pressure. Complete excision of one kidney was also not sufficient to produce any elevation of pressure; (4) The increase of arterial tension reached its height within a few days of the completion of the operative procedures, and thereafter it tended to return to normal. The rapidity with which the change occurred appeared to be roughly proportional to the amount of functioning renal tissue remaining.

To summarise the foregoing types of experiment, it may be concluded that the balance of opinion is against the occurrence of hypertension after unilateral or bilateral nephrectomy. On the other hand, hypertension probably follows the progressive reduction of renal tissue either by excision or ligation.
ligation, especially when the minimum amount of renal tissue necessary for life is being approached. It is apparently as yet undecided whether this hypertension requires for its production the presence of necrotic renal tissue, although the balance of evidence is rather in favour of Paessler and Heineke (1905) and Janeway (1913) who maintain it is independent of such a factor.

Another line of approach is by means of ureteral ligature. This method was employed as far back as the middle of last century by Beckman (1857) who ligated one ureter in the guinea pig and thought that after four months he got some left ventricular hypertrophy; this, however, was a purely qualitative observation. Rautenberg (1910) blocked one ureter in rabbits for three weeks and then removed the obstruction and excised the sound kidney. He observed the animals for periods up to 21 months and he found that there was hypertension up to 170 mm. Hg., the controls being 122 mm. Hg. He observed pressure by inserting a cannula into the carotid artery. Hartwich (1930), as already mentioned, found hypertension to follow both unilateral and bilateral ligature of the ureters. From the scanty available/
Production of Chronic Renal Venous Stasis
by the Method of Bell & Pederson.
available evidence it would therefore appear that the ureteral blockage is followed by hypertension in the experimental animal.

Katzenstein (1905) claimed to get a slight rise in blood pressure after incomplete occlusion of the renal vessels and after temporary complete occlusion by which he claimed to produce thrombosis of the smaller renal arteries. Further experiments in this direction made by Müller and Maas (1911) who embolised the kidney extensively with paraffin, failed to demonstrate a rise in blood pressure. Alwens (1916) also failed to confirm Katzenstein's observations but he was able to produce small rises in blood pressure by compressing the kidneys in oncometers, using an amount of external pressure approximating or surpassing the aortic blood pressure. The same amount of pressure applied to the lower extremities was without influence.

Bell and Pederson (1930), working with rabbits, has succeeded in producing a pronounced chronic venous congestion in one kidney. Their procedure was to isolate carefully the left renal vein and surround it by an aluminium wire. The wire was then tightened until only a small lumen was left in the vein/
vein. This caused the kidney to become swollen and cyanotic. In those cases in which the constriction of the vein was too great, a haemorrhagic infarct developed and the experiment failed. However, in those in which the wire was tightened to the correct degree the kidney was removed from its fossa and then surrounded with a thin membrane which was drawn round the hilum. The organ was then replaced and secured by sutures. The presence of the membrane was found necessary as, otherwise, a collateral venous circulation was rapidly established and the congestion subsided in a few days. The net result of this operation was to get the kidney in a state of distension with tense capsule and greatly increased resistance to the circulation of blood. After the operation the blood pressure was found to rise slightly and to remain well above the normal level for about two months, after which time it returned slowly to normal, pressures as high as 260 mm. being recorded. The kidney inside the membrane showed very striking changes. At the end of two months the capsule of the kidney had become enormously thickened and there was pronounced atrophy of the parenchyma. The authors considered the capsule thickening to be due to the irritation of the/
the surrounding membrane, while atrophy of the parenchyma was attributed to the extreme passive congestion. The hypertension was considered to be due to the obstruction to the renal circulation and was considered to be analogous to the processes frequently observed in human renal disease.

Loesch (1933) published a report similar to this piece of work. He used dogs and produced renal damage by means of an operative procedure in which he mobilised the kidneys and brought them to the surface so that they were only covered by skin. When the wounds had healed he found it possible to apply pressure to the renal pedicle. This was done for periods ranging from 10 to 25 minutes on one to three successive days. In one group the operation was unilateral, while in another group both sides were subjected to this procedure. In the former group the hypertension that resulted ranged from 18 to 46 mm. Hg., whereas in the latter group hypertension was more pronounced, ranging from 46 to 64 mm. Hg. The pathological changes were essentially ischaemic in nature.

A very impressive report of work along lines similar to those just described comes from the Western Reserve University. The authors are Goldblatt/
Renal Artery Clamp of Goldblatt.
Goldblatt, Lynch, Hanzal and Summerville. They employed dogs which had had the carotid artery exteriorised by the method of van Leersum. Blood pressure was determined daily for at least two months before and for from three days to fifteen months after the constriction of both renal arteries by means of a very ingenious clamp devised for the purpose. This clamp permitted the degree of constriction of the vessel to be varied and increased at will. In some of the animals the degree of constriction was made great from the beginning; in others it was made moderate at first and subsequently on one or more occasions. They found that constriction of one renal artery was followed by a moderate or slight rise of blood pressure, which tended to return to normal after a few days. However, following the production of bilateral renal ischaemia, the systolic blood pressure rose to a varying degree in all of the animals.

Following the constriction of the second renal artery, very high values indeed were recorded (260 mm. Hg.). They made no attempt to reduce or remove the accessory circulation through the capsule of the kidney/
kidney; this, they consider, was the probable cause of the tendency of the hypertension to subside in those animals in which the constriction was only moderate at first. In two animals in which the constriction of both arteries was made almost complete from the first, the rise of blood pressure which followed was accompanied by the development of uraemia, which rapidly proved fatal. In these animals blood urea nitrogen, non-protein nitrogen, and creatinine increased, and the urea clearance and phenolsulphonphthalein diminished until death. In the remaining animals which survived for many months the only renal function test which indicated renal damage was the urea clearance. The nitrogen constituents of the blood remained within normal limits. In one animal that had a persistently elevated pressure for 15 months following severe constriction of both main renal arteries, the urea clearance remained reduced throughout to about 50 per cent of the mean value obtained during the control period. As a control measure in one dog the splenic and both femoral arteries were greatly constricted at different times before the clamps were applied to the renal arteries, but no hypertension occurred until/
until after the renal arteries of this animal had been constricted. In one animal the right suprrenal body was removed, the left suprarenal body was denervated and its medulla mechanically destroyed, and the left major and minor splanchnic nerves were sectioned. The blood pressure showed no significant change until after the renal arteries were constricted, when a moderate rise promptly occurred and persisted. This appears to eliminate the possibility of the suprarenal playing a part in the phenomenon.

The examination of the kidneys of three animals which had had hypertension for some time indicated that ischaemia can produce changes in the glomeruli, tubules and vessels of the kidney. Gross infarction was not observed in these kidneys and massive necrosis was not present. They regarded the changes in the tissues of the animals with persistent hypertension and without signs of uraemia as "abiotrophic rather than necrobiotic". They concluded that necrosis of renal substance was not a necessary condition for the development of elevated blood pressure in these animals, and that the hypertension/
hypertension was attributable to the atrophy. These results, they consider, go far to explain the occurrence of the hypertension of benign nephrosclerosis in man. On the other hand, the great hypertension and renal failure that accompanies almost total constriction of both arteries resembles the condition of nephrosclerosis malignum.

Radiation by X-rays.

The search for some means of producing experimental nephritis comparable with lesions occurring in man has embraced the use of X-radiation. Linser and Baerman (1904) reported that albuminuria followed radiation of kidneys. Buschke and Schmidt (1905) by exposing the kidneys of rabbits directly through a surgical incision, obtained necrosis of both cortex and medulla in two animals 7-8 weeks after radiation. Schulz and Hoffman (1905) also made a study of the action of Rontgen Rays on renal tissue. By means of a surgical operation they brought kidneys of rabbits to the skin surface and radiated them, protecting the surrounding tissue with lead screens. Kidneys were examined from 1 hour to 48 days after irradiation. They did not combine this procedure with functional or vascular studies.
The early changes obtained were haemorrhage and transudation throughout the interstitial tissue, with congestion of the glomeruli and in some instances complete tubular disintegration. In the later experiments the interstitial tissue increased in proportion until, at the end of 48 days, the picture was that of a typical chronic interstitial nephritis. The blood vessels were stated to show muscular hypertrophy and swelling. Shortly afterwards Warthin (1904) furnished evidence of renal damage produced by prolonged radiation in both the experimental animal and in cases of leukaemia. In the human cases lesions consisted of epithelial degeneration and calcification. He sounded a note of warning with regard to the use of X-rays in leukaemia. He suggested repeated urinary examinations, and he stated that in a nephritic, serious aggravation of the lesion might result from X-radiation. Hall and Whipple (1919) considered that renal injury as the result of X-rays was only of relatively slight grade and was not a constant finding. Thereafter there was no further work done in this for some years. Indeed, about 1924 several observers advocated deep X-ray therapy as a successful treatment for oliguria and anuria.
anuria. Martin (1924) while attempting to injure by X-rays the suprarenal through a surgical incision, irradiated the upper pole of the kidney and produced marked interstitial changes. Schulz and Hoffman (1905) working with rabbits produced acute, sub-acute and chronic varieties of renal damage by the use of X-rays. The doses they used were 200 Kilo-volts, 4 milliamperes at 50 mm. skin distance for a period of 2 hours. Their results demonstrated quite definitely that a chronic interstitial nephritis can be produced by exposure of the kidney through the abdomen without extensive damage to other organs.

One of the most complete pieces of work in this respect was that of Hartmann, Bolliger and Doub (1926), and no apology need be made for quoting their work in some detail. They used dogs. Care was taken to ensure that the animals were thoroughly healthy before being selected for experimental purposes. The X-rays were applied over an area varying from 10 - 15 cm. square, with the costavertebral angle as the approximate centre. Time of application varied from 15 - 60 minutes with 200 Kilovolts at 30 milliamperes infiltration, with 1 mm. copper and 1 mm. aluminium at 50 cm. skin distance.

They used 26 dogs in their experimental series.
Of those, 8 died within 5 days of exposure, 18 were observed for periods ranging from two to thirteen months, and 14 came to autopsy. Not a single animal failed to show kidney damage both from a functional and histological standpoint. In one half of the surviving animals there was diarrhoea, often blood-stained, for several days, but at post-mortem only a few depressed scars were found, usually in the duodenum or first portion of the jejunum. After the acute stages the dogs usually gained weight and remained fat and sleek until the terminal stage.

The effects upon the kidney were divided into three groups, - acute, sub-acute, and chronic.

(a) The acute stage was observed from 3 - 5 days after irradiation, the kidney was larger, the capsule stripped readily leaving a smooth dark red surface with here and there recent extravasations of blood on the cortical surface. The cut surface was much congested, the glomeruli standing out as deep red dots. Microscopically, blood vessels were dilated and engorged and intertubular haemorrhages were numerous. The glomerular tufts were swollen and engorged and in some instances infiltrated. The tubular epithelium was swollen, granular, and
in places necrotic.

(b) The sub-acute stage was observed from 4 - 8 weeks after irradiation. Deaths were not wholly due to renal insufficiency but to various complications such as distemper, intestinal obstruction and two from suppurative nephritis. The kidneys of those which did not develop the suppurative process were smaller than normal. The capsule was thickened, the surface showed the cortex to be greyish in colour, and granular, with some distortion of the normal dots and striae. Microscopically, the glomeruli were comparatively well preserved but the tufts were often smaller than usual and there was an increase of pink-staining hyaline stroma at the expense of the capillaries. In a few instances the capsular spaces contained hyalinised thrombi. The tubules were widely dilated and were lined with flattened, atrophic epithelium.

(c) The chronic stage was observed in animals surviving longer than 3 months and dying of renal insufficiency. In several of those the renal artery was thickened and rigid although not calcified. The capsule was grey and opaque; on stripping it carried away some of the cortex with it. The cortical surface was smooth, dark red in colour, and of an opaque/
opaque glassy translucence. There were numerous slightly depressed grey areas. On section there was a dense fibrous surface, greyish red in colour. The cortex was thin. Microscopically, the most striking finding was the increase in interstitial tissue. The glomeruli appeared more numerous than normal and were relatively undamaged. Bowman's capsule was thickened; some of the glomerular tufts showed hyalinisation and atrophy. Blood vessels were thick-walled and many showed endarteritis. The tubules were distorted and the lining epithelium was composed of flattened, desquamated or vacuolated cells. In several areas tubules were entirely replaced by connective tissue.

Among the functional changes noted were albuminuria and cylindruria; phthalein excretion was depressed till in the final stages no dye was excreted at all. There was marked polyuria with low specific gravity in the sub-acute stages, and oliguria or anuria with low specific gravity in the final stages. Changes in the blood chemistry were typical, showing retention of nitrogen beginning in the sub-acute stages and increasing during the experiment, reaching great heights during the terminal/
terminal stages. Blood pressure was observed throughout by the auscultatory method of Allen. It was found that the blood pressure was slightly increased, the diastolic rising relatively more than the systolic. In some instances in the final stages the systolic pressure reached 230, the diastolic 150.

In those animals showing marked increase in blood pressure, retinal changes similar to the so-called albuminuric retinitis were found. Oedema was not observed in any of the animals. A marked diminution of alakli reserve was noted in the terminal stages. Clinically the terminal stages were characterised by vomiting, convulsions, and coma.

This piece of work probably represents one of the nearest experimental approaches to a condition comparable with chronic glomerulo-nephritis in man. It is unfortunate that the method of production of the lesion is probably quite beyond the reach of workers on this side of the Atlantic because, as far as can be ascertained, there is probably not more than one or two X-ray tubes of sufficient power in this country, and even if there were, the cost of maintenance would be prohibitive.

**Nephrotoxic Substances.**
With regard to the method of producing hypertension by injection of nephrotoxic substances one of the most complete pieces of work was that of Dominguez. He was not primarily investigating hypertension but was examining the arteriosclerotic lesions that result from the injection of uranium nitrate. He did not confine himself to this substance alone but he also investigated the effects of lead, radium, and vanadium. In this series of experiments he employed 66 rabbits. Carotid loops for the purpose of measuring the blood pressure were made in 48 animals by the method of van Leersum. Satisfactory loops were obtained in 32. Uranium was injected under the skin on the back of the rabbits in concentrations of 1 in 5,000, 1 in 500, and 1 in 200 once or twice weekly. Radium was injected subcutaneously in the form of a solution of radium bromide in such strength that 1 c.c. contained from $1 \times 10^5$ to $1 \times 10^4$ mgm. of radium bromide per c.c.

Vanadium chloride was given subcutaneously in a 1 in 1,000 solution. Lead was given in two forms - lead carbonate smeared on carrots by mouth daily, and lead acetate in watery solution in 1 - 4 per c.c. by/
by stomach tube once or twice weekly. He also used combinations of uranium with radium, and radium with vanadium. A considerable portion of his paper is devoted to considerations of the vascular lesions produced as the result of these injections. Renal lesions were variable according to amount of uranium and duration of experiment. These varied from the general swelling of an acute nephritis to an extraordinary shrinkage of the kidneys with fine granulation of the subcapsular surface. The most common lesion found consisted of reduction of cortical thickness with obliteration of the normal striations combined with fine greyish mottling of the external surface without granulations and congestion of the bases of the pyramid. Microscopically these lesions correspond to necrotic changes of the tubules with and without calcification.

The effects on the blood pressure were variable. The systolic blood pressure was measured almost daily on 32 rabbits. Towards the end of the investigation, however, measurements were made every 4 days as the results had been so consistently negative. Changes in blood pressure did not occur under/
under the influence of radium, lead, or vanadium. Changes in blood pressure were not found in acute intoxication caused by uranium. On the contrary, under the influence of injections of uranium the blood pressure in general was lowered. The fact that in intoxication caused by uranium the blood pressure either is unaltered or becomes lower, has the following significance when brought into correlation with pathological lesions:—(1) Severe arteriosclerosis may be produced without elevation of the blood pressure; (2) Nephritis of all degrees from acute swelling down to the stage of granular contraction caused by uranium is not accompanied by hypertension; and (3) Calcification of the interlobular arteries in the kidney and pronounced calcification of Bowman's capsules are not necessarily accompanied by hypertension.

Rafsky, Bernhard, and Rhodenburg (1935) used uranium as a nephrotoxic agent and they noted in addition to the other well-known changes a definite hypertension, pressure being estimated by the abdominal band method of McGregor. They also investigated the pressor effects of a variety of other substances. Repeated injections of cholesterol/
cholesterol into rabbits produced a moderate hypertension. Protein putrefaction products such as putrescin and tyramin were devoid of pressor effect. A variety of amino-acids were tried and of these aspartic acid was found to be the most active. The substance was given in 200 mgm. doses for 25 - 180 injections. Albumin and casts usually appeared in the urine within 4 to 6 weeks of the first injection; nitrogen retention was not noted. At autopsy the kidney lesion was that of a glomerulonephritis with congestion of the tufts and exudation into the capsular space. Pictures of the lesion are published and they do not indicate a lesion in any way comparable to the human form of the disease. The blood pressure rose steadily during the course of injections reaching a maximum in from 4 to 6 months. These workers further noted a curious change in the serum proteins of animals receiving aspartic acid injections; there was decrease of the basic amino nitrogen fraction of the serum proteins and increase of the monoamino nitrogen fraction, and a rise in the ratio of the monoamino nitrogen to the basic amino nitrogen.

A long and detailed report of various experimental
experimental methods of producing a diffuse damage to the vascular system was published by Ruhl (1929). He found that repeated injections of lead acetate produced a very pronounced and sustained hypertension which led to left ventricular hypertrophy. The nature of the histological changes in the organs was so variable that he concluded that the hypertension was attributable to a spasm of the arterioles. He succeeded in producing a definite hypertension by means of repeated subcutaneous injections of digalen. A further series of rabbits was subjected to a prolonged series of injections of ephetonin (Merck's synthetic ephedrine) and a definite hypertension resulted. There was cardiac hypertrophy, and in the kidney and spleen arteriolar thickening was visible. The fact that long continued medication with ephedrine causes hypertension was confirmed by Rothschild (1931) who reported a series of experiments in which rabbits developed an ephedrine hypertension. Blood pressure was estimated by the carotid loop method.

Masugi (1933) working first with Theodore Fahr in Hamburg and later in Japan has employed an entirely new method for the investigation of
experimental nephritis and hypertension. A separate section of the thesis is devoted to his work and to personal results using this method.

Experimental oxalate nephritis is also considered in a later section.

Hypertension by Section of the "Hochdruckzügler."

Since the middle of the last century it has been known that there was a system of depressor nerves that arose in the aortic arch and coursed centrally, and that these constituted the afferent limb of a reflex arc governing blood pressure. However, it has not been until the last ten years that the brilliant researches of Hering (1927), Koch (1928), Nordman (1929), and Mies (1929) have demonstrated that this reflex arc had additional afferent end organs situated in the commencement of the internal carotid artery, an area which has come to be called the carotid sinus.

Experimenters were not long in realising that they now had at their disposal a valuable method for producing chronic hypertension, - that is the section of these depressor nerves from the aortic arch/
arch and the carotid sinuses. As long ago as 1910 Bruns and Genner claimed to have produced cardiac hypertrophy and aortic thickening in four dogs that were made to work daily on the treadmill following section of the aortic nerves. Of course, in these experiments nothing was done to the carotid sinuses. Velluda (1927) failed to confirm this work although he omitted the forced exercise from his experiments. Hirsch and Thorspecker (1912) described arteriosclerosis in rabbits following the repeated injection of adrenalin after section of the aortic depressor nerves; similar changes were not noted in intact animals. Koch and Mies (1929) have extirpated the aortic and sinus nerves in the rabbit in two stages separated by an interval of 10 to 14 days. Thereafter the animals were allowed to survive for periods ranging from 14 to 511 days. In the case of some of these rabbits the blood pressure was determined terminally by inserting a cannula into the carotid artery in the unanaesthetised animal. In just over 50 per cent of the rabbits pressures over 150 mm. Hg. were obtained, the normal pressure being regarded as 100 mm. Hg. In other rabbits repeated determinations were made under anaesthesia
by a bloodless method; in 75 per cent of these pressures over 140 mm. Hg. were obtained. In the case of the operated animals in which the blood pressure was not found to be raised, section of the vagi in acute experiment produced a pronounced elevation. The inference drawn from this last observation was that in these animals a significant proportion of the depressor fibres had been running in the vagus trunk. The chief pathological changes noted in these animals were in the aorta, the heart, and the kidney. The aortic lesion consisted of medial degeneration with secondary calcification. Diffuse or discrete patches of fibrosis were observed in the heart and a certain degree of destructive change was observed in the glomeruli of the kidney. Sclerosis was noted at times in the pulmonary arteries. The extent of the aortic lesion was thought to be proportional to the degree of hypertension which developed. Miess (1929) has observed a chronic elevation of the blood pressure in the rabbit following denervation of the carotid sinuses alone. Koch (1928) and also Heymans (1931) have produced chronic hypertension in dogs by similar methods.
Goormaghtigh (1929) of Ghent published a paper on experimental renal sclerosis in the rabbit. His method of producing the hypertension was the denervation of the carotid sinuses and section of the aortic depressor nerves. He states that in a rabbit killed 2½ months after denervation he found changes in at least one glomerulus out of ten. These changes consisted of a thickening and hyalinisation of the framework of the tuft. The cells lining the tuft were swollen and showed signs of active division. In the case of another rabbit he states that uniform thickening of the stroma of the tuft was visible in 90 per cent of the glomeruli. The thickened tissue was hyalinised and he noted that the swollen endothelial cells appeared to become more and more absorbed in the collagenous material. He noted that the capillaries appeared to "twine" in the surface of the hyaline masses. Their lumen, however, showed a progressive reduction apparently as the result of the slow closure of the afferent artery and the pressure of the hyalinisation. Some adhesion finally became established between the tuft and the capsule.

With regard to the interstitial tissue,
Goormaghtigh noted some proliferation in the neighbourhood of the glomeruli. Changes in the tubules were negligible.

With regard to vascular lesions, the existence of which Nordman denied, he states that the afferent glomerular arteries showed changes comparable with those seen in human hypertension. These consisted of hyperplasia of the plain muscle cells to such an extent as to give them an appearance resembling epithelial cells. There was also reduplication of the internal elastic lamina. In his conclusions Goormaghtigh considered that chronic arterial hypertension obtained by denervation of the carotid sinuses, and section of the aortic depressor nerves caused a progressive hyalinization of the glomeruli. This change is evident at the end of 3½ months, arterial pressure having been maintained 15 mm. above the normal. This lesion is accompanied by a slight degree of interstitial fibrosis, especially in the neighbourhood of the glomeruli.

Hyperplastic and degenerative lesions are also present in the renal arterioles and he considers that these are the cause of the glomerular lesions and that the changes are strictly comparable.
comparable with those seen in essential hypertension of man.

This paper is not very convincing. His personal observations appear to be based on some four rabbits. The method of recording the blood pressure is not mentioned.

Kremer, Wright and Scarff (1933) published a report dealing with the effects on the tissues of chronic arterial hypertension. They used rabbits, and the removal of the depressor nerves was carried out in two stages with an intervening interval of about one month. Owing to the difficulty of identifying the aortic nerve with certainty in the rabbit, and the fact that a certain number of aortic fibres run in the vagus trunk, they removed the vagus nerve together with the aortic branch on the right side and the aortic nerve alone on the left side where it is larger and more easily recognised. In many experiments they also removed the cervical sympathetic chain as some of the aortic fibres often run in close association with it. In order to record pressure they constructed carotid loops by the method of van Leersum. Their results are grouped under two heads, -(a) Blood pressure changes/
changes, and (b) Pathological changes. Both groups of results merit reporting.

(a) Blood pressure changes: The total number of animals used was 57. In the case of 32 of these satisfactory carotid loops were obtained. The proportion of successful results they considered to be low, because, as they pointed out, in addition to the normal risks and difficulties associated with preparing the loop there was the additional danger of the unilateral denervation, which in itself is attended with considerable risk to life. In view of present results with this method we consider their results more a matter for congratulation than apology. Seven of the animals either tore through their loops or died of infection before more than a few determinations had been made. The remaining 25 animals had the blood pressure studied during the interval between the first and second operations. Satisfactory readings could not be obtained until 2 weeks after the first operation as time had to be given for the loop to heal. After this unilateral operation they found the average pressure to be 115 mm. Hg., which figure indicates a slight persistent elevation of the blood pressure.

In/
In 24 animals with loops, denervation was carried out on the second side; 15 of these did not survive the second operation, either dying immediately or after a short interval, the cause of death being apparently heart failure associated with pulmonary oedema. In 4 others the loop was injured or the neck became infected. Five animals survived in a satisfactory state. They found that the hypertension did not develop to its full extent till three or four weeks after the second operation. The average pressure in this series was 160 mm. Hg.

(b) Pathological Changes: The material investigated was obtained from 42 animals of which 22 were killed at various times after the double operation, 12 after the single operation, and 8 were normal control rabbits from the same source of supply. The changes found after the double operation were as follows:

Aorta: The earliest lesion apparent to the naked eye consisted of a minute bleb slightly raised above the inner surface of the vessel. The next stage appeared as a thickened whitish area in the vessel wall. The gross lesions consisted of thick white calcified plaques which maintained the curve of the vessel/
vessel wall. These changes were most frequent in the ascending portion of the aorta and progressively less frequent towards its termination. Microscopically, the changes were seen to be confined to the media. Early lesions showed small foci of degeneration in the media, the muscle fibres being replaced by hyaline material; the elastic fibres appeared smaller but were otherwise unaffected. There were also present in the degenerated area cells with palely staining finely granular nuclei and ill-defined cell outline. These cells they considered to be either degenerate muscle cells or histiocytes. The more extensive lesions showed fragmentation of the elastic fibres, increase in the mononuclear cells, and early calcification. The advanced lesions showed a central structureless calcified area. The intima showed no gross changes except in those instances in which the medial lesion was situated immediately beneath its coat, when there was some fibrosis. They also considered that, broadly speaking, there was some relationship between the duration of the survival period and the extent of the lesions.

Heart: The heart was sectioned in 12 animals killed after/
after the double operation and in 6 after the single operation. The hearts of those which had undergone the double operation all showed a patchy fibrosis which was most marked in the wall of the left ventricle. They considered that there was some left ventricular hypertrophy.

**Other organs:** Sections were taken of other organs of several of the animals. Apart from the question of vascular hypertrophy on which no definite conclusions have been reached, no significant changes have been found. The renal lesions described by Goormaghtigh as occurring in this condition were not encountered.

“Hypertension by Intra-cranial Interference”. Dixon and Heller (1932) reported that a prolonged and severe hypertension could be produced in dogs by injections of kaolin into the cisterna cerebello medullaris. Blood pressures as high as 180 mm. Hg. were observed. This has been confirmed by Braun (1933), Braun and Samet (1934) who found that chronic hypertension thus produced in dogs could be terminated by bilateral renal denervation. Högler, Überrack, Zell and Falta (1934) also produced hypertension by Kaolin injection, and like Braun and Samet/
Samet they found that it appeared to be dependent upon intact renal innervation. They further found that prior denervation of both suprarenal glands did not prevent the onset of the hypertension. All workers appear to be unanimous in attributing the hypertension to the occurrence of raised intracranial pressure.

Various forms of acute experimental interference with the base of the brain have been studied. Albert (1934) injected defibrinated blood into the third ventricle of dogs and noted a prompt hypertension. Jaegher, Bogaert, and Adelbert (1935) stimulated the hypothalamic region in dogs and noted hypertension. They took care to eliminate asphyxial and diaphragmatic spasm by prior vagotomy and phrenicectomy combined with artificial ventilation of the preparation. They found that the hypertension did not occur if both sympathetic chains and major splanchnic nerves had been cut. Ergotamine tartrate was also noted to prevent the occurrence of the hypertension. These results would appear to attribute the hypertension to sympathetic stimulation.

Hoff and Urban (1933) reported observations on a series of dogs in which they produced a localised/
localised damage to both corpora mammillaria. In the five animals which survived the operation there was a profound hypotension which lasted for a few days. This was followed by a progressive hypertension which was observed for as long as six months. In their discussion they state "Wir haben im Corp. mam. des Hundes ein wichtiges Zentrum für die Regulation des Gefäßsystems."

On has personally noted the association of sustained hypertension with tumours of the third ventricular region. There would, therefore, appear to be a certain amount of evidence pointing towards the existence in the hypothalamic region of some centre controlling blood pressure. It is hoped in the near future to further investigate this point.
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EXPERIMENTAL OXALATE NEPHRITIS.

Kobbert and Kussner (1879) showed that oxalates exerted a pronounced specific action on the kidneys. The principal changes that they have noted in non-fatal cases were albuminuria and oliguria in the human subject, and in their experimental work they found similar changes in animals. The principal pathological changes that they noted were enlargement and pallor of the kidneys with some faint white streaking, specially in the boundary zone of the medulla.

Microscopically the streaking was found to be due to a heavy tubular deposit of crystals of insoluble calcium oxalate. They did not notice any glomerular changes. The following year Frankel (1880) carried out further observations on oxalate nephritis. Using the subcutaneous route of administration he was unable to produce either albuminuria or oliguria in rabbits, although he used doses similar to those employed by Kobbert and Kussner. However, in rabbits killed after 3 - 4 days he noticed the same deposition of oxalate crystals in the tubules. Koch (1881), during the course of investigation into the effects of oxalate on/
on the tissues in general, noted the same pale striping along with pallor and enlargement of the kidneys.

Murset (1885) injected subcutaneously doses of potassium oxalate of such an order as to produce death in $1\frac{1}{2}$ - 5 days. He did not consider that there were any obvious naked-eye changes, but microscopically he found glomerular and tubular changes. The glomeruli showed swelling of the tuft and thickening of both the glomerular and capsular epithelium, along with an albuminous exudate in the spaces. In the tubules there was commencing necroses of the epithelium with swelling, vacuolation, karyolysis and karyorhexis. The lumina contained crystals and granular masses of hyaline casts.

Some years later Ebstein and Nicolaier (1897) attempted to produce granular kidneys in rabbits by the administration of oxalic acid. They found albuminuria but their naked-eye and microscopic observations did not differ materially from earlier workers. Experiments lasting up to one year failed to produce granular kidneys.

Increase of crystals in the renal tubules after oxalate administration was again noted by Veitinghoff-Scheel/
Veithninghoff-Scheel (1901). From 1901 - 1924 there were apparently no further attempts to use oxalates as nephropathic irritants. The reason for this is difficult to understand, unless it may be that, by routes other than the intravenous, their effects are rather variable.

It may be noted that other members of the di-carboxylic group have been found to damage the kidneys. Baer and Blum (1907) found that tartrates administered subcutaneously would prevent the development of phlorhydrazin glycosuria. They attribute this effect to necrotic lesions of the convoluted tubules produced by tartrates.

Shaw-Dunn, Haworth, and Jones (1924) published a report of a very careful research into oxalate nephritis. They observed (a) the effects of large doses, single or sub-divided, and (b) the result of successive doses over a period. Their main experimental animal was the rabbit. In the more acute experiments, results showed that oxalate damaged principally the convoluted tubules, necrosis being clearly established in from 1 - 2 hours after administration of the dose. After 8 - 24 hours necrotic changes were well established, the tubular lumina/
lumina being packed with glomerular detritus. By the end of 48 hours regeneration is apparent, especially in the descending limbs of the loops of Henle. Apart from these changes localised to the first convoluted tubules, they found more diffuse later changes in all the tubules, one of the most prominent being diminished affinity for eosin stain. They concluded that the brunt of the lesion falls on the lower segments of the first convoluted tubules, the probable explanation being that (1) the bulk of the oxalate is excreted probably within a few minutes of injection, and that it is excreted by only a few glomeruli; this is in agreement with the fact that all the glomeruli in a kidney are not at work at one time as was shown by Khanolkar, and has subsequently been shown more definitely by Richards. (2) The lower segments of the first tubules are not mainly affected because the filtrate becomes more concentrated at that level owing to re-absorption of water. These experiments tend to show that actual crystallisation of the oxalate plays a comparatively unimportant part in the damaging of the kidneys, as these changes must have been produced long before crystallisation could have occurred. The crystals must be comparatively inert/
inert. The glomerular changes were comparatively slight in these experiments, although after 24 hours haemorrhages into the tuft were abundant. The somewhat late appearance of haemorrhages these workers concluded to be due to a preliminary capillary stasis with haemorrhages as the stasis passed off. Changes in the other organs consisted of pulmonary oedema and some ascites, probably due to capillary damage.

**Results of Successive Doses over a Period.**

They found the results to agree well with variations dependent on the duration of the experiments. The changes were most pronounced in the epithelium of the first convoluted tubules. In the earlier stages the changes were degenerative, with desquamation and some regeneration of epithelium. In later lesions the tubules tended to become shrunken and atrophic with, by this stage, definite thickening of the basement membrane. Crystals of calcium oxalate were now apparent in the tubules, although once again a definite impression was formed that these played no essential part in the pathological process. The loops of Henle and second convoluted tubules showed little change, and the glomeruli showed no definite structural alterations, although/
although these workers make the observation that they must have had an increased permeability to albumin. This is of interest because it anticipates by some years the modern view that the increased glomerular permeability is the fundamental error in albuminuria and especially in oedema of the nephrotic variety. No haemorrhagic changes were noted. They refer the functional changes to destructive lesions in the first convoluted tubules.

The principal microscopic observation in those more chronic experiments was the pallor and swelling of the kidneys. These they attributed to interstitial oedema which by pressure upon the vessels caused pallor.

A very valuable section of the paper by Shaw-Dunn, Haworth and Jones is devoted to consideration of the doses which can be used in oxalate nephritis. They concluded that intravenous dosage was by far the most useful method owing to the constancy of the results obtainable. Using the subcutaneous route Koch (1881) estimated the fatal dose for an average rabbit as 0.25 grms. per Kilo, and Veitinghoff-Scheel (1901) as 0.26 grms. per Kilo. Intramuscularly, Gates regarded the lethal dose as in the region of 0.2 grams per Kilo. Apparently with oxalate there is/
is a comparatively narrow margin between producing no specific renal effect at all, and killing the animal.

The three main points which they regarded as important in striking this happy mean were adequate dilution of the salt, a sufficient interval between doses, and careful observation of the animal's symptoms. They found that 40-50 mgms. per Kilo may be given intravenously in 0.5 per cent solution with little risk of producing serious symptoms. In the production of their acute experiments they found it necessary to give the largest possible dose in the course of a few hours. This means 40-50 mgms. per Kilo as a first dose, followed by 15 mgms. per Kilo in 1½ - 2 hours, and a smaller dose two hours later. As an example of what can be done with smaller doses they quoted an experiment in which the rabbit received 20 mgms. per Kilo of sodium oxalate in 0.5 per cent solution four times daily at intervals of 2½ hours. This was carried on for four days without the animal losing much weight or failing to take food.

It might be permissible to quote verbatim the conclusions of those three workers. "(1) By intravenous/
intravenous administration of soluble oxalate to rabbits it is possible to produce a well-marked nephritis in which the largest convoluted tubules are specifically damaged. The deposition of calcium oxalate crystals in the renal tubules plays no important part in this lesion. (2) If sufficiently large doses of oxalate are administered within the space of a few hours necrosis is produced in some of the tubules and the glomeruli are temporarily put out of action owing to stasis. This is followed by abundant haemorrhage from the tissues. (3) Successive administration of similar doses produced an obvious subacute catarrh in the first convoluted tubules without structural changes in the glomeruli. (4) In oxalate nephritis the kidneys are swollen and oedematous and become very pallid, owing to pressure on the blood vessels. This has probably the significance of diminution in the circulation through the organs."

Browne and Dodds (1928) during an investigation of the etiology of accidental haemorrhage observed the effect of a prolonged series of injections of oxalate on the kidneys. They found that, naked eye, the kidneys were enlarged, congested.
congested and oedematous, while microscopically there was a very pronounced chronic interstitial change. This was diffuse but more pronounced in the region of the first convoluted tubules. They did not observe any inflammatory reaction in the form of cellular infiltration. In the areas affected all but a few degenerate remaining tubules had disappeared and had been replaced by connective tissue. A large proportion of the remaining tubules were dilated with pronounced reduction in the type of epithelium. Beyond slight dilatation there were no changes in the glomeruli.

In the study of the effects of nephro-toxic agents one has always to consider the possibility that the lesion observed may have been due to spontaneous nephritis rather than to the nephro-toxic agent employed. Nazum, Elliot and Priest (1932) have made a careful study of spontaneous nephritis as found in the rabbit. Broadly speaking, they distinguish two types - (1) That in which there is a diffuse interstitial fibrosis with some round-celled infiltration, the glomerular and tubular changes being slight; and (2) that form in which there are small, wedge-like zones of dense interstitial/
interstitial proliferation with atrophy of the tubules but with no definite glomerular change. It is noteworthy that no lesion resembling human glomerulo-nephritis was observed.
PERSONAL EXPERIENCE OF OXALATE NEPHRITIS.

It was considered that it would be of interest to attempt to ascertain if experimental oxalate nephritis were associated with hypertension. To this end a stock of animals with successful loops was accumulated. In the course of this there accumulated a number of rabbits whose loops were for some reason or other unsatisfactory. Three of these were employed for the induction of oxalate nephritis without, of course, any reference to any change in blood pressure that might result. It was obviously desirable to have some experience of the method before employing it on valuable animals with successful loops.

Experiment 1. Rabbit "706". On the 22:2:34 a right carotid loop was torn without any haemorrhage.

A 1 per cent solution of anhydrous sodium oxalate was used throughout. 1 cc. of this solution = 6.6 mgm. H2C2O4.

Urea in the urine was estimated by the Hypobromite method. Before the experiment began it was ascertained that the urine contained no abnormality. Diet was reasonably constant.

Day/
<table>
<thead>
<tr>
<th>Day</th>
<th>Urine 24 hrs. vo.</th>
<th>Urea Gm.%</th>
<th>Blood Urea N.</th>
<th>Oxalate</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73 cc.</td>
<td>2.6</td>
<td></td>
<td></td>
<td>Nil.</td>
</tr>
<tr>
<td>2</td>
<td>39 cc.</td>
<td>4.5</td>
<td></td>
<td></td>
<td>Nil.</td>
</tr>
<tr>
<td>3</td>
<td>50 cc.</td>
<td>3.0</td>
<td>16mgm.%</td>
<td>3cc.I.V.</td>
<td>Nil.</td>
</tr>
<tr>
<td>4</td>
<td>64 cc.</td>
<td>1.8</td>
<td>Plus.</td>
<td></td>
<td>Nil.</td>
</tr>
<tr>
<td>5</td>
<td>32 cc.</td>
<td>2.4</td>
<td>Plus.</td>
<td></td>
<td>Nil.</td>
</tr>
<tr>
<td>6</td>
<td>55 cc.</td>
<td>3.0</td>
<td>4cc.I.V.</td>
<td>Nil.</td>
<td>Nil.</td>
</tr>
<tr>
<td>7</td>
<td>53 cc.</td>
<td>1.7</td>
<td></td>
<td></td>
<td>Nil.</td>
</tr>
<tr>
<td>8</td>
<td>55 cc.</td>
<td>3.3</td>
<td></td>
<td></td>
<td>Nil.</td>
</tr>
<tr>
<td>9</td>
<td>33 cc.</td>
<td>3.6</td>
<td>33mgm.%</td>
<td>5cc.I.V.</td>
<td>Trace.</td>
</tr>
</tbody>
</table>

Apparently the rabbit sustained an injury to the spinal cord during this injection as it developed a paraplegia with some retention, which rendered unreliable the urinary output figures. It died on the 12th day of the experiment.

Post-mortem: On opening the abdomen the bladder was seen to be distended. Kidneys were slightly enlarged and were pallid. On section there was marked pallor with lack of differentiation between the cortex and the medulla. There was a faint suggestion of some pale streaks in the boundary zone.

Other organs presented no abnormality. The vertebral column was removed en bloc and fixed in 10 per cent formol. There was no obvious evidence of injury.

Histology: It was only the first convoluted tubules.
tubules that showed marked change. There was a marked poverty of staining with eosin. The cells were swollen and they showed a fine droplet content which was apparently not fatty in nature. The nuclei in some instances showed karyorhexis. Changes in the other tubules were negligible. The glomeruli showed no definite change. Staining with Azan (Heidenhain's azo-carmine) showed no increase of connective tissue either in the glomeruli or in the interstitium. There was slight congestion of the interstitial tissue. There was no suggestion whatsoever of any pyogenic infection of the organ despite the nervous involvement of the bladder.

The heart showed no microscopic change.

**Experiment 2. Rabbit "811".** On the 29:1:34 a right carotid loop was prepared. The loop became fibrosed and quite useless for the purpose of taking blood pressure.

<table>
<thead>
<tr>
<th>Day</th>
<th>Urine 24 hrs.</th>
<th>Urea Gm.%</th>
<th>Blood Urea N.</th>
<th>Oxalate</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81 cc.</td>
<td>2.4</td>
<td></td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>65 cc.</td>
<td>1.9</td>
<td>12 mgm.%</td>
<td>4 cc.</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>65 cc.</td>
<td>1.3</td>
<td></td>
<td></td>
<td>Plus</td>
</tr>
<tr>
<td>4</td>
<td>165 cc.</td>
<td>1.5</td>
<td></td>
<td></td>
<td>Plus</td>
</tr>
<tr>
<td>5</td>
<td>70 cc.</td>
<td>1.7</td>
<td>26 mgm.%</td>
<td>5 cc.</td>
<td>Plus</td>
</tr>
<tr>
<td>6</td>
<td>59 cc.</td>
<td>1.5</td>
<td></td>
<td></td>
<td>Plus</td>
</tr>
<tr>
<td>7</td>
<td>33 cc.</td>
<td>2.7</td>
<td>24 mgm.%</td>
<td>5 cc.</td>
<td>Trace</td>
</tr>
<tr>
<td>8</td>
<td>50 cc.</td>
<td>3.3</td>
<td></td>
<td></td>
<td>Trace</td>
</tr>
<tr>
<td>9</td>
<td>45 cc.</td>
<td>2.7</td>
<td></td>
<td></td>
<td>Nil</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>30 mgm.%</td>
<td>5 cc.</td>
<td>Nil</td>
</tr>
<tr>
<td>11</td>
<td>40 cc.</td>
<td>1.6</td>
<td></td>
<td></td>
<td>Plus</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>30 mgms%</td>
<td></td>
<td>Plus</td>
</tr>
<tr>
<td>13</td>
<td>68 cc.</td>
<td>2.6</td>
<td></td>
<td></td>
<td>Trace</td>
</tr>
<tr>
<td>14</td>
<td>65 cc.</td>
<td>-</td>
<td>34 mgm.%</td>
<td>5 cc.</td>
<td>Trace</td>
</tr>
<tr>
<td>15</td>
<td>Animal died, having been in poor condition for some days. No terminal fits.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post/
Post-mortem: There was no excess of free fluid in the serous sacs. The kidneys were slightly swollen and pale. On section they were very definitely pallid. There was a lack of distinction between the cortex and the medulla. There was some fine stippling in the region of the boundary zone.

The liver was rather pale but otherwise there was no abnormality in the other organs.

Histology: Kidneys: The changes in the kidneys were comparatively slight. The convoluted tubules showed some swelling of the epithelium. The protoplasm in some places had lost some of its affinity for eosin. The protoplasm showed a certain amount of fine vacuolation which does not appear to be fatty in nature. These changes were more pronounced in the tubules immediately underlying the capsule. The glomeruli showed the capillaries to be rather empty and there were many swollen, darkly-staining nuclei present in the tuft. Sections stained with Azan and Weigert-Van Gieson showed no increase in the interstitium or in the connective tissue of the glomeruli. The vessels were healthy.

Liver: This organ showed some cloudy swelling, probably/
Oxalate Nephritis.
probably quite a genuine observation in this case as the autopsy was performed within a few minutes of death.

Heart: The heart showed no microscopic change.

Experiment 3. Rabbit "826"; female. A right carotid loop was prepared on the 10:1:34. The loop fibrosed and was torn without serious haemorrhage on the 8:2:34.

<table>
<thead>
<tr>
<th>Day</th>
<th>Urine 24 hrs. vol.</th>
<th>Urea Gms.%</th>
<th>Blood Urea N.</th>
<th>Oxalate</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44 cc.</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>53 cc.</td>
<td>3.3</td>
<td></td>
<td>14 mgm.%</td>
<td>3 cc.</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>33 cc.</td>
<td>2.5</td>
<td></td>
<td>3 cc.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29 cc.</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>62 cc.</td>
<td>2.7</td>
<td></td>
<td>10 cc.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>115 cc.</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>58 cc.</td>
<td>1.5</td>
<td></td>
<td>34 mgm.%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>26 cc.</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Animal died at 10 a.m. having had fits just before death.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Post-mortem: There was no excess of free fluid in the serous sacs. The kidneys were slightly larger than normal. On section the surface was pallid with poor differentiation between the cortex and medulla.

The other organs appeared healthy.

Histology: Kidneys: The first convoluted tubules/
tubules showed advanced degenerative changes. The cells were swollen and there was extensive vacuolation of the protoplasm which was not fatty in nature according to fat stains. The lumen of the tubules were reduced to mere chinks. The nuclei showed a certain amount of karyorhexis. Once again these changes were much more advanced in the zone immediately underlying the capsule. The glomeruli showed dilated capillaries. All the cortical vessels showed definite dilatation. The Azan and Weigert-Van Gieson sections did not show any increase of the connective tissue of the organ.

Heart: There were occasional collections of small round cells, apparently lymphocytic in nature.

Liver: There was a pronounced degree of cloudy swelling.

Discussion.

These three isolated experiments served to give some experience in the use of sodium oxalate as a nephrotic agent. The results correspond in the main to those described by Shaw-Dunn, Haworth and Jones. In the last experiment which was the most acute there was very definite droplet degeneration of the first convoluted tubules with little other change except/
except the congestion of the glomeruli and the interstitium. The second example showed comparatively slight tubular changes, once again confined to the first convoluted tubules. The glomeruli here, however, were somewhat ischaemic and the nuclei were very prominent and dark-staining and gave the impression of some nuclear increase. All degenerative changes are more pronounced in the subcapsular zone. This is not due to coincident post-mortem autolysis as normal control animals showed no such changes. The blood urea never failed to show a rise after the injection of oxalate. The output of urine and the urea content of the urine was so variable as not to permit of any deductions being made. If these results are to be of any value it is necessary to adhere to an absolutely standard diet. This was not done in these preliminary experiments.
Personal Work on the Relationship  
between Oxalate Nephritis and Hypertension.

The production of renal damage and hypertension by injection of some nephrotoxic substance seemed more desirable than any of the operative procedures that had been used. Owing to the conflicting nature of the evidence as to the hypertensive effect of the substances investigated it was decided to determine whether there was any hypertension associated with experimental oxalate nephritis.

Methods: A stock of chinchilla rabbits was accumulated, each provided with satisfactory functioning carotid loops according to the method of van Leersum. As far as possible, pressures were recorded twice daily and blood pressure records were taken hourly during the twenty-four hours immediately after the first injection of oxalate. These readings were controlled by a similar series of observations made during the control period. Fourteen animals were employed. Eight of these were placed on a relatively dry diet of oats and bran with an occasional cabbage leaf with no water. The other six received a cabbage diet with water ad lib. One animal in each group served as a control. Twenty-four/
four hour urine volumes were observed and the urine examined for albumin, urea content, specific gravity, and occasionally blood.

After a prolonged period of control lasting several weeks the animals were given a course of sodium oxalate intravenously, the injection being repeated every second day. The initial dose was 50-60 mgms. sodium oxalate, which was raised after two injections to 80-100 mgms, depending on the weight of the animal. The control animals received equivalent volumes of normal sodium chloride solution intravenously to eliminate the possibility that mere bulk of fluid altered the blood pressure. Blood urea nitrogen readings were made on all animals during the control period and on those animals which survived a period of three weeks of oxalate injections.

Results: The average normal systolic pressure in the group of 14 rabbits was 96 mm. Hg. Two of the rabbits fed on a dry diet were not included in the final statistical analysis as in one of them there was evidence of a pre-existing spontaneous nephritis, while in the other a fault developed in the loop which rendered a reliable series of observations impossible. This resulted in a reduc-
Blood Pressure Graph of Individual Rabbit.

Blood Pressure Graph of Control Group.
reduction of the "dry" diet group to six. It is worthy of note that the example of spontaneous nephritis showed a degree of hypertension considerably greater than the others.

In order to render the observations comparable the pressures of each animal were calculated as percentages of its average normal pressure during the control period. In the case of the animals on a dry diet the mean pressure for the six days immediately prior to the first oxalate injection was 98.3 per cent ±0.3 per cent. Following the injection of oxalate there was a prompt elevation of pressure which reached a maximum of 135 per cent on the eighth day. The mean pressure of the twelve days immediately succeeding the first injection was 122 per cent ±1 per cent. By the end of this period pressure had subsided to an average level slightly in excess of that of the control period although it now showed a pronounced instability. Statistically, the elevation of the pressure was highly significant. The hourly observations showed that the pressure began to rise within nine hours of the first oxalate injection.

The animals fed on the "wet" diet showed a similar/
The interrupted curved regression lines connect the polynomial values which were calculated by the usual statistical methods.
similar though less pronounced elevation of pressure. The pressure of the last six days of the control period was 98.6 per cent ± 0.3 per cent. The maximum pressure of 128 per cent was attained on the fifth day; thereafter the pressure gradually fell to an average level considerably in excess of the control period. There was again pronounced instability of the pressure. The mean pressure of the first twelve days of the injections was 111.5 per cent ± 0.7 per cent. Again this elevation was highly significant. The appearance of the hypertension was not so prompt as in the "dry" group, a full day elapsing before the pressure commenced to rise.

A statistical comparison of the degree of hypertension observed in the two groups showed that of the dry group to be significantly greater.

The control animals showed no variation in pressure as a result of the repeated injections of normal saline.

The volume of urine passed during the period of oxalate nephritis showed no significant variation from that of the control period in either group. Albuminuria was constantly observed. Haematuria was only occasionally noted.

Blood urea nitrogen observations indicated a moderate/
moderate elevation reaching as high as 60 mgms. per cent in one animal.

No animal died before the period of hypertension had passed off. At the end of three weeks after injections had commenced, seven animals receiving oxalate survived. There was 15-20 per cent reduction in weight of the animals during the injection period. There were no changes in weight such as would suggest the occurrence of oedema.

Seven animals which had received oxalate injections have been submitted to complete post-mortem examination. Histological examination was made of the various organs. The only organs presenting abnormalities were the kidneys. On naked eye examination these were enlarged and pale. Sections were stained by haematoxylin, by Heidenhain's method, and for fat. In general these showed the changes associated with oxalate nephritis such as have been already described by Dunn et alia, and earlier in this report, consisting of intense swelling of the convoluted tubules with degeneration and necrosis. Beyond congestion, the glomeruli showed no definite alteration. In the case of the rabbit which had received injections over a period of five-and-a-half months there was a slight but definite/
definite increase of the interstitial tissue. This was diffuse, with here and there patches of more pronounced fibrosis. However, there was no evidence of glomerular destruction although in some instances there was thickening of the capsule. These changes are similar though much less in degree than those noted by Browne and Dodds.

**Discussion.**

From these results it would appear that repeated injection of sodium oxalate into rabbits results in a definite hypertension of approximately twelve days duration. Thereafter, pressure shows a pronounced instability although it never reaches such a height as during the initial period of hypertension. This hypertension appears sooner and is more pronounced in the case of animals fed on a "dry" diet. It is hardly surprising that there is to be found a difference in the reaction of animals on such diets as the conditions of renal function are so widely dissimilar. Those on a "dry" diet produce scanty concentrated urine rarely in excess of 50 cc. per diem, with occasional periods of anuria as long as thirty-six hours; whereas those on the "wet" diet pass as much as 500 cc. and rarely
less than 150 cc. Possibly the more pronounced hypertension in the "dry" group is due to an element of functional overstrain of the kidneys. Chanutin and others have shown that a renal damaging agent associated with functional overstrain results in more pronounced hypertension.

Whether or not this hypertension is of renal origin the albuminuria, the nitrogen retention, and the histological picture indicate that at least it is associated with renal damage. Although it is impossible at present to advance any evidence as to the mechanism by which the pressure is elevated, it might be suggested that it is in some way associated with glomerular ischaemia resulting from the pressure exerted by the swollen tubular epithelium. The one common factor that is shared by most varieties of renal disease associated with hypertension is some interference with glomerular circulation. It has to be realised, however, that owing to widely dissimilar etiology and histology experimental oxalate nephritis sheds little light on the problem of human glomerulonephritis. Rather is it to be hoped that this experimental production of hypertension may provide a basis for the investigation of the, as yet, unsolved relationship/
relationship between the renal damage and hypertension.

The following experiments were, therefore, performed in order to investigate the role of efferent nerve impulses in renal hypertension:

(1) The pressure changes following bilateral nephrectomy in the rabbit were observed; (2) Bilaterally nephrectomised rabbits, whose survival period is usually at least five days, were subjected to injections of oxalate; and (3) A series of rabbits have had one kidney removed and the remaining kidney deprived of its nerve supply. There were subjected to oxalate injections.

It was hoped by these means to shed some light on the part played by the efferent renal nerves in renal hypertension.
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Personal Observations on the Blood Pressure in Bilaterally Nephrectomised Rabbits both with and without Oxalate Injections.

In order to determine whether or not oxalate injections produced hypertension as the result of renal damage it was decided to observe the blood pressure in bilaterally nephrectomised rabbits which had had oxalate injections.

The Effect on the Blood Pressure of Bilateral Nephrectomy

Ten animals with prolonged blood pressure control had both kidneys removed under nembutal and ether anaesthesia. The abdomen was opened through a mid-line incision. The peritoneum overlying the kidneys was incised and then an incision was made through the true capsule of the kidney over the outer curvature. The capsule was then rapidly stripped down to the hilum. The pedicle was clamped and doubly ligatured and the organ then excised. It was found that stripping the true capsule was the quickest way of gaining access to the pedicle as it provided the best line of cleavage in the peri-renal tissues. The pedicle has to be particularly carefully secured on the right side owing to the shortness of the renal/
Graph of Bilaterally Nephrectomised Animals.
renal vein. The abdominal wall was then closed by two tiers of continuous silk sutures. The animals were running about within a few hours of the operation.

The excised kidneys were examined histologically to exclude nephritis. Blood pressure readings were resumed within a few hours of operation and numerous readings made daily during the survival of the animals. In order to provide a control group, three animals were subjected to an operation of approximately equivalent severity, namely, unilateral nephrectomy, and on them similar observations were made.

Results: Two animals did not survive operation more than twenty-four hours and are not included in the results. Of the remainder three survived three days, one four days, one five days, two six days and one a full week. In these eight animals there was a rapid recovery from the operation and until a few hours before death they took food, and in general appeared fairly normal. Occasional twitchings were noted although nothing in the nature of a generalised convolution was seen. In order to render the observations comparable, the pressures in each animal were calculated as percentages/
percentages of the average normal pressure during the control period. A composite graph was then constructed and a statistical analysis made. The mean pressure for the ten days immediately prior to bilateral nephrectomy was $99.6 \pm 0.7$ per cent. The average pressure of all readings taken after operation was $78.2 \pm 2.2$ per cent. Reference to the graph shows that there was a gradual fall in pressure during the survival period, although the average pressure never fell below 70 per cent. At no time in any animal did the pressure taken after operation rise above its control. A statistical comparison of the mean pressure before and after nephrectomy showed that the readings taken after operation were significantly lower. The control animals with unilateral nephrectomy showed no significant variation.

**Discussion.**

This series of experiments was considered necessary because one was able to find records of only three satisfactory researches on this point and these were not in agreement. Mosler (1912) carried out bilateral nephrectomy on 13 rabbits, taking one blood pressure observation before operation and one 48 hours afterwards. In 11 animals there/
there was hypertension. Backman (1916) carried out similar experiments on cats. Apparently he used only two animals, neither of which showed hypertension. Hartwich (1929-30) removed both kidneys in 10 dogs. In six there was an average elevation of 10 mm. Hg. He considered this to be within the range of experimental error and concluded that bilateral nephrectomy did not produce hypertension.

Having shown that bilateral nephrectomy is associated with a fall in blood pressure, the next procedure was to try the effect of oxalate on such animals.

The Effect of the Administration of Oxalate to Bilaterally and Unilaterally Nephrectomised Rabbits.

Four animals after a satisfactory period of blood pressure recording were subjected as before to bilateral nephrectomy. All were dead on the evening of the third day following operation. They had received one injection of oxalate as soon as they recovered from the anaesthetic and another on the third day. Each injection consisted of 2 cc. of a 2 per cent solution of sodium oxalate. The average pressure before operation was 99±0.9 per cent and during the survival period 84±2.0 per cent, i.e. a significant/
significant fall.

Two animals had one kidney removed and received oxalate injections immediately after operation. They showed the usual hypertensive response associated with oxalate nephritis.

Thus the administration of oxalate to bilaterally nephrectomised animals merely hastened death without causing a rise in blood pressure.

Up to this point it has been shown that (1) removal of one or both kidneys does not cause hypertension; (2) the injection of sodium oxalate into animals (a) with intact kidneys, (b) unilaterally nephrectomised, results in hypertension; (3) the injection of sodium oxalate into animals with no kidneys results in no rise of blood pressure. It was therefore considered that the hypertension which results from the injection of sodium oxalate into animals is of renal origin.
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RENAL DENERVATION.

An essential preliminary to any operative interference with the renal nerve supply is a review of the present state of knowledge of renal nerves. Loeb (1905-6) suggested that the hypertension of certain forms of renal disease might be produced as the result of autonomic impulses originating in the glomeruli and influencing the vaso-motor centre. However, he did not advance any proof of this possibility. Indeed, one finds that the possibility of efferent nerves from the kidney hardly receives mention. The nerve supply of the kidney is derived from the renal plexus which originates in the aortic plexus and is situated alongside the renal artery and in the hilum of the kidney. Its fibres originate in the coeliac ganglion and in the aortic plexus, and it receives contributions from the lowest splanchnic nerve and the adrenal plexus. It may also receive a branch from the lesser splanchnic nerve.

Langley and Anderson (1895) and Jost (1914) have stated that small branches from the lumbar sympathetic trunk enter the renal plexus. The vagus also/
also gives branches to this plexus. From the renal plexus the nerve fibres enter the kidney alongside the renal vessels and follow them to their finest ramifications, small fibres being found in the smallest afferent and efferent arteries and even on the capillaries. There have been described nerve fibres between the cells of the tubules and immediately underneath the capsule. Fibres in the pelvis and calyces are usually medullated, while those in the parenchyma of the organ are non-medullated. It has further been observed that fine nerve fibres enter the kidney with small arteries which penetrate the fibrous capsule. Apparently no fibres enter the kidney from the wall of the ureter.

Before giving an account of the experimental work on the innervation of the kidney one may summarise the modern view as to the part played by nervous influence on renal function. Renal nerves are almost entirely vaso-motor in function. The splanchnic nerve conveys numerous vaso-constrictor and some vaso-dilator fibres to the kidney, but the vaso-constrictor action predominates over the vaso-dilator with the result that stimulation of the splanchnic trunk lessens the blood flow from the kidney and diminishes the amount of urine secreted.
On the other hand, section of the splanchnic nerves increases the amount of urine which assumes the characteristics observed in other forms of diuresis. There is no evidence to suggest that the splanchnics contain any renal nerve fibres other than vaso-constrictors and vaso-dilators. The vagus does not appear to contain vaso-motor fibres for the kidney, and it exercises no direct influence over the secretion of urine. With the exception of Asher and Pearce (1913) it has been the universal opinion that there are no direct secretory nerves to the kidney. Those two workers attempted to show that the vagus contains specific secretory fibres for the kidney, in the same way as the chorda tympani contains secretory fibres for the submaxillary gland. Howell has summed up the consensus of opinion as follows:

"The majority of purely physiological experiments upon direct stimulation of the nerves going to the kidneys are adverse to the theory of secretory fibres, the marked effects obtained in these experiments being all explicable by the changes produced in the blood flow to the kidneys."

Furthermore, the modern theory of urinary secretion elaborated by Cushny (1917) and supported and/
and confirmed by many investigators, especially A.N. Richards (1922), does not invoke the necessity or admit the existence of secretory nerves.

It is well known that the renal secretion is subject in some degree to reflex nervous influence. Inhibition results from cooling of the skin, while increase of secretion follows warming of the skin. Muller, Peterson and Rieder (1930) carried out experiments which appear to show that the renal vessels contract in sympathy with the cutaneous blood vessels, the result being that exposure to cold was associated with a renal vaso-constriction. They further observed that this vaso-constriction appeared to diminish the resistance of the kidneys to harmful agents. Large injections of bacillus coli, followed by chill, resulted in the prompt appearance in the urine of albumin, erythrocytes, and bacteria. However, in animals with one kidney previously denervated the kidney continued to excrete normal urine after the injection of bacteria in spite of chill; whereas the normally innervated kidney showed prompt evidence of damage. Renal activity is also subject to reflex inhibition as the result of impulses arising elsewhere in the urinary system/
system. Irritation of the bladder or ureter by instrument or calculus may cause complete anuria; stone in the kidney, kinking or compression of the ureter, ligature or compression of the renal plexus by a pack after nephrectomy may cause reflex anuria or oliguria on the other side through a reflex spasm of the renal arteries. In other conditions the pathological changes may be on one side while pain or other subjective symptoms are referred to the other side. This phenomenon constitutes the "reno-renal" or the "crossed renal reflex". The pathway of this reflex is believed to be in the splanchnic nerve and the transference from one side to the other in the individual segments of the spinal cord.

Turning now to consider the actual experimental work on kidney innervation one finds that it is based on the fundamental discovery of Claud Bernard (1851) that section of the sympathetic nerves on one side of the body resulted in increased temperature on that side. The following year Brown-Sequard (1857) showed that the sympathetic nerves were true vaso-constrictors. Eight years later Bernard found that section of a splanchnic nerve on one side resulted/
resulted in increased secretion of urine on that side. This has been confirmed by Eckard (1869), Knoll (1872), Klecki (1879), Vogt (1898), Grek (1912), Rhode and Ellinger (1913), Jungmann and Meyer (1913), and other more recent investigators. It appears that the innervation of the kidney is not essential to life; in fact, it has been shown that excretion continues apparently indefinitely after all connection between the central nervous system and the kidneys has been divided. Carrel and Guthrie (1906) transplanted the kidneys of one dog into another and removed the kidneys of the latter without fatal results. In this case the transplanted kidneys must have carried on their functions for many days without any guidance from the central nervous system. Lobenhoffer (1913) claimed that a transposed kidney was able to meet the ordinary demands of life. He severed the renal pedicle and transposed the kidney to the splenic vessels and made a detailed study of the function of the transposed kidney. Zaaijer (1914) reported that a dog lived for six years after its single kidney had been transposed to the iliac vessels. Dederer (1918) transposed the left kidney of a dog to/
to the neck and two weeks later did a right nephrectomy. The dog remained alive and well for more than four months after the operation. The transposed kidney excreted phenolsulphonephthaline rapidly and there was a marked increase in the urine secreted by the kidney after removal of the other. Dederer also did a homo-transplantation of a kidney and an ovary from one dog to another of the same litter. The homo-transplantated kidney functioned for 26 days until the dog died of distemper. Phenolsulphonephthaline injected intravenously appeared in the urine from the transplanted kidney in 2 minutes 40 seconds. Pathological examination showed that the organ reacted to the distemper in a similar manner to the animal's own kidneys. These transplantation experiments have this advantage that the kidney is certainly deprived of its nerve supply. Quinby (1916) claimed that stripping the renal vessels of nerve fibres gives no reliable results because a few of the nerve fibres are actually within the vessel walls. His procedure was to remove the kidney on one side in dogs, then re-implant that organ by anastomosing the severed vessels and the ureter. The other kidney was removed in 5 days to 2 weeks after the primary/
primary operation and it was found that the re-implanted kidney was capable of maintaining life indefinitely. He operated on 43 dogs, 16 of which survived in a condition suitable for further experiment. In his first series one kidney was transplanted and the other left in position. Some days after the primary operation the ureters were brought out through the flanks and the urines collected and compared. It was found that the denervated kidney showed increased excretion both of fluid and of salt persisting for 10-14 days. It was noticed that the absence of nerves abolished the reflex inhibition seen after handling the ureters. The normal side showed inhibition, while none was apparent on the denervated side. In a second series nephrectomy was done on the normal side 5 days to 2 weeks after the preliminary operation. Elimination from the single denervated kidney of salt, lactose and phenolsulphonephthaline was then compared to that from a normal dog in which a single nephrectomy had been done. In each case the results were comparable, suggesting the absence of secretory fibres to the kidneys. In a third series he tested the response of the denervated kidney to hypertonic solution/
solution of sodium chloride, urea, and caffeine, and concluded that the reactions of the normal and the denervated kidney were practically identical for the same diuretics. Bellido (1917) of Barcelona denervated one or both kidneys in the dog by cutting the nerve fibres running along the renal vessels. Denervation in one kidney resulted in moderate polyuria from that side. In saline diuresis the excess of secretion from the denervated kidney was found to be more pronounced. He found that if both kidneys were denervated there was a polyuria lasting as long as two weeks followed by gradual suppression and death in coma. Bominghaus (1923) denervated both kidneys at the same operation on six animals. His method was to tear away the fibres of the renal plexus and to paint the renal pedicle and hilum with a concentrated solution of phenol. All the dogs lived until autopsy nine months later, these results being in direct disagreement with those of Bellido. One of the most impressive modern researches on the subject is that of Marshall and Kolls (1919). They studied the result of renal denervation in relation to diuresis in urinary secretion. After the preliminary operation their observations were made with/
with the dog under paraldehyde anaesthesia. In some cases the splanchnic nerve was sectioned, in others the renal plexus. The urine was collected from the ureters through small glass cannulae an hour before and an hour after giving diuretics. They found that denervation of a kidney caused increased flow of urine on that side with a relative reduction but total increase of solids. These changes persisted for months. Their general conclusion was that the changes noted in the urine from the denervated kidney were caused solely by vaso-dilatation with a corresponding increased blood flow from the kidney. In support of this hypothesis they produced changes of an opposite nature by constriction of the renal artery. They found that where a denervated kidney was secreting three times as much urine as the normal kidney they could once more be restored to equality of function by paralysing the splanchnic on the normal side. They found no evidence of the existence of a secretory inhibitory action of the splanchnic nerve other than could be produced by vaso-motor action.

Some mention may be made of those who have subscribed to the hypothesis of specific secretory nerves/
nerves to the kidneys. Rhode and Ellinger (1913) and Jost (1914) have suggested that the splanchnic nerves carry fibres which act directly on renal cells. Ellinger (1921) maintained that the splanchnic fibres accompanying the renal artery exert an inhibitory influence on the secretion of water and the solid constituents of the urine. He claimed that the vagus and splanchnic affect the water excretion in the same way as that of the solid constituents in the opposite way. Könnicke (1923) concluded from his experiments on dogs that a denervated kidney produced urine which in itself appears normal in composition, but is deficient in saline content and amount as compared with the urine from the other kidney. His further conclusion was that a kidney deprived of its nerves sufficed to maintain life in normal conditions but failed when greater demands were made upon it. His results concerning the amount of urine excreted by the denervated kidney are off-set by those of Bominghaus (1923).

Kusakari (1930) observed the rate at which phenolsulphonephthaline was excreted by the kidneys before and after denervation. It was found that both water/
water and the drug were excreted in equal quantities by the kidneys with intact nerves. The splanchnic nerve was then sectioned on one side. This resulted in increase in the output of urine on the operated side with, however, no alteration in the rate at which the drug was excreted. He interpreted this observation as indicating the influence of splanchnic impulses on the resorbtive activities of the renal tubules.

Braun (1933) and Braun and Samet (1934) have performed some extremely interesting and it may well be fundamental experiments. They produced hypertension in dogs by means of injection of a suspension of Kaolin into the cerebello-medullary cistern. They then denervated the kidneys and found that the hypertension disappeared within 24 hours. In a further series they produced hypertension by excision of the carotid sinuses and section of the aortic depressor nerves. Once again the hypertension was promptly terminated by denervation of the kidneys. In some cases they performed the denervation prior to the injection of Kaolin and it was found that this entirely prevented the occurrence of hypertension. Their method of denervating/
denervating the kidney consisted of opening the abdomen, isolating the renal pedicles, scraping the vessels and painting with 5 per cent phenol. They suggest that hypertension may be produced by efferent autonomic impulses from the kidney. It is surprising to find that renal denervation terminates experimental hypertension of extra-renal origin.
Operation of Renal Denervation.
Personal Work on Renal Denervation in Oxalate Nephritis.

The animals were first provided with successful carotid loops, and were subjected to a control period of blood pressure observation.

The effect of oxalate injections in rabbits with one kidney removed and the other denervated.

Methods: Although denervation of the renal artery in the rabbit is not a particularly difficult operation it is very easy to damage the renal vein, especially on the right side. It was soon decided that it would be best to remove the right kidney entirely and to denervate the left, which has a long and easily accessible pedicle. After a suitable control period the animals were operated on under nembutal and ether anaesthesia. The left kidney was entirely separated from its fascial attachments to the posterior abdominal wall and the fat around the renal pelvis and ureter cleared off. The artery, after being gently separated from the vein for a distance of \(\frac{5}{8}\) inch or so, was held on a flat metal surface and gently scraped with the edge of a sharp knife. With experience it was possible to remove a considerable portion of the coats of the vessel without puncturing it. When the vessel was satisfactorily cleaned it swelled. The ureter was then also gently scraped. Attempts/
Effect of oxalate nephritis on unilaterally nephrectomised animals (a) with remaining kidney denervated, (b) with remaining kidney normal.
Attempts to scrape the vein usually ended in failure. It was considered unnecessary to apply alcohol or carbolic acid to the scraped artery.

Of 18 animals thus operated on 13 survived and were suitable for the next part of the experiment. On the morning of the ninth day following denervation a course of injections consisting of 2 c.c. of a 2 per cent solution of sodium oxalate was commenced, doses being administered every second day. As a control two animals had unilateral nephrectomy performed and received oxalate on the ninth day following.

**Results:** As before, pressures were calculated as percentages of the average normal pressure during the control period. In the thirteen animals successfully denervated the average pressure during the fourteen days prior to operation was $100 \pm 0.44$ per cent. In the same group, during the eight days after denervation and before oxalate administration, the pressure was $100 \pm 0.49$ per cent. Following the injection of the oxalate, pressures were measured over a further period of fourteen days; they averaged $101 \pm 0.55$ per cent. It is thus seen that the injection of sodium oxalate into animals with a solitary/
solitary denervated kidney produces no hypertension. In only one animal of the thirteen was there a significant rise of pressure, but this did not significantly influence the aggregate.

The two non-denervated control animals had an average pressure during the fourteen day control period of $100 \pm 0.9$ per cent. During the eight days following unilateral nephrectomy and before oxalate the pressure was $102 \pm 0.9$ per cent. The average pressure during the fourteen days following the injection of oxalate was $128 \pm 1.3$ per cent. These animals, therefore, had the typical hypertension response which follows the induction of oxalate nephritis.

**Discussion:** The previous results indicated that hypertension was associated with oxalate nephritis in the rabbit. An investigation into the mechanism of the nephritis was some attempt to prove that the hypertension was directly due to the renal damage inflicted by the oxalate. It was to this end that the first two groups of experiments detailed above were devised. Firstly, it was observed that bilateral nephrectomy resulted in a definite and progressive fall in blood pressure. It was further determined/
determined that the animals survived in apparent health for an average period of five days. Secondly, similar bilaterally nephrectomised animals receiving oxalate immediately after operation showed no material difference in pressure from those of the first group. On the other hand, animals subjected to unilateral nephrectomy and receiving oxalate under the same conditions showed a hypertensive response fully as great as those with normal kidneys. It was, therefore, considered justifiable to assume that the hypertension in oxalate nephritis was the result of the renal damage. It was then shown that removal of one kidney with denervation of the other entirely abolished the hypertension of oxalate nephritis.

Conclusions: (1) A progressive fall in blood pressure has been found to follow bilateral nephrectomy in the rabbit.
(2) The hypertension in oxalate nephritis would appear to be due to renal damage.
(3) Denervation of the kidney abolishes the hypertension of oxalate nephritis in unilaterally nephrectomised rabbits.
Experimental Glomerulo-Nephritis
and Hypertension.

The results obtained with oxalate nephritis were extremely interesting and suggestive, but there was the inherent objection that oxalate nephritis is not a glomerulo-nephritis and bears no resemblance to human glomerulo-nephritis. It was, therefore, decided to attempt to repeat the same experiments using a method of producing renal damage the characteristics of which closely resembled the human form of the disease.

Review of Possible Methods.

The etiology of diffuse glomerulo-nephritis is still obscure. It has been recognised, since the work of Loehlein (1910, 1917), that it occurs as a sequel to an infection frequently streptococcal. At the same time there is no evidence to show that the condition is due to the direct action of organisms upon the kidneys. Urine culture in glomerulo-nephritis fails to reveal the streptococcus, blood culture affords no evidence of septicemia, and kidney sections do not show streptococci. It has therefore come to be believed that the renal lesion is/
is the result of soluble toxic products of organismal activity. The observation of Trask and Blake (1924) that the urine in some cases of scarlatina gave a skin reaction similar to that produced by the "Dick Toxin" would appear to establish the fact that the products of the streptococcus are eliminated by the kidney. Longcope (1929) advanced further evidence which points to the same conclusion. Glomerulonephritis, however, cannot be ascribed to the direct action upon the kidney of some harmful substance produced by the streptococcus, as the renal lesion does not occur at the height of the infection, but occurs some three or four weeks later. This curious time relationship led Schick (1907), and later, von Pirquet (1911), to compare the specific complications of scarlatina, such as glomerulonephritis, with serum disease occurring one or two weeks after the injection of horse serum. They concluded that post-scarlatinal nephritis might be regarded as a manifestation of heightened sensitivity of the individual to the infection. The term "allergy" was applied by von Pirquet (1911) to this particular state.

The further problem that arises is the determination/
determination of the factors which decide the incidence of renal damage. Longcope (1929) and his co-workers observed that the skin-reactivity of individuals suffering from glomerulo-nephritis to filtrates of cultures of haemolytic streptococci is very much greater than in controls such as uncomplicated tonsillitis. According to Freidmann and Deicher (1928) the blood of patients with post-scarlatinal glomerulo-nephritis contains a greater concentration of antibodies than does the blood of non-nephritic cases of scarlatina at a comparable stage of convalescence. The clinical and bacteriological evidence would appear to indicate that the occurrence of diffuse glomerulo-nephritis is due either to a greatly exaggerated and acquired sensitivity of the kidneys to the products of the streptococcus or to the toxic products of an exceptionally vigorous antibody-antigen reaction such as might occur during the period of regression of the infection.

One of the principal difficulties which beset the clinical investigator into the pathogenesis of glomerulo-nephritis is the comparatively small number of early cases which enter hospital. Too often this stage either passes unrecognised or is deemed not sufficiently
sufficiently severe to warrant reference to hospital. Furthermore, the course of the disease frequently extends over many years, thus making the complete study of an individual case a somewhat difficult procedure. In view of these difficulties it is natural that strenuous attempts should have been made to reproduce the disease in experimental animals. Until very recently this has proved to be an insoluble problem. Hadfield and Garrod (1934) stated that "certainly no disease resembling chronic glomerulo-nephritis has yet been produced." It has been found comparatively easy to produce severe renal tubular damage with a diversity of agents, such as sodium oxalate and uranyl nitrate, but these changes neither clinically nor pathologically resemble human glomerulo-nephritis.

Before discussing recent attempts to produce glomerulo-nephritis it is desirable to summarise the clinical and pathological features which should characterise an experimental lesion that could be regarded as comparable to the human lesion. Clinically, the urine should show albuminuria, haematuria, and cylindruria, while some degree of oedema should be present and a transient but definite hypertension/
hypertension should occur. Pathologically, the early stages should be predominantly glomerular, consisting of swelling and proliferation of the glomerular cells with ischaemia of the capillary loops. These changes should progress to proliferation of the epithelium of Bowman's capsule with obliteration of the capsular space, the formation of "epithelial crescents" and the final replacement of the glomerulus by fibrous tissue. In the early stages the convoluted tubules should show cloudy swelling with some fatty degeneration. The fully established nephritis should show some interstitial fibrosis which in the later stages is principally focal in distribution.

Among the more recent and partially successful attempts to reproduce glomerulo-nephritis may be mentioned that of Longcope (1913) who subjected dogs, rabbits and other animals to repeated injections of egg albumen and horse serum. In some cases these measures produced round-celled infiltrations in the cortex with cellular proliferation and other changes suggestive of glomerulo-nephritis, in the neighbouring glomeruli. This work was repeated by Pentimalli (1929), Vaubel (1932), and others. Lukens and Longcope/
Longcope (1931) injected suspensions of heat-killed streptococci directly into the renal artery of the rabbit. In a proportion of the animals glomerular lesions were observed, especially in those previously subjected to an acute localised infection produced by the intracutaneous injection of living streptococci. Once again, however, the glomerular lesions were not diffuse and did not closely resemble the human lesion. Blackman, Brown and Rake (1931) injected rabbits with an autolysate of Type I pneumococci. Renal lesions resulted which were claimed to resemble human glomerulonephritis; their illustrations, however, hardly support their contention. Duval and Hibbard (1926) produced what they termed a glomerulonephritis by the injection of living scarlatinal streptococci into immunised rabbits. Similar injections into non-immunised animals were without result. Furthermore, they obtained similar positive results by injecting the streptococcal endotoxin, liberated either by bacteriolysis in the peritoneal cavity of the immune animal or by bacteriolysis in vitro with the activated homologous immune serum. Once again the illustrations are not convincing. In connection with/
with this work the interesting suggestion has been advanced that glomerulo-nephritis is due to the liberation of excessive amounts of endotoxin. Bell and Clawson (1931) reported an isolated experiment on a monkey. Over a period of four years this animal was subjected to injections of a suspension of a culture of streptococcus viridans. The animal eventually developed albuminuria and haematuria, but no hypertension or ascites, and death ultimately occurred apparently from uraemia. At autopsy there was present a diffuse glomerulo-nephritis characterised histologically by increase in the capillary endothelium with thickening and proliferation of the layers of the capillary basement membrane. The foregoing reference demonstrates the partial success which has attended the use of methods based on immunity reactions in the production of experimental glomerulo-nephritis.

Ever since Lindemann (1900) injected guinea pigs with an emulsion of rabbits' kidney and obtained a serum which on injection into rabbits produced albuminuria and renal degeneration, it has been known that it was possible to prepare sera pathogenic to the kidney. However, no use of this phenomenon in the/
the investigation of renal disease appears to have been made prior to the work of Masugi (1933, 1934). He found that, by subjecting rabbits to a series of parenteral injections of a suspension of rat's kidneys, the serum of the rabbits eventually developed the power of producing glomerulo-nephritis when injected intravenously into rats. Masugi (1934) repeated this work on rabbits, using ducks as the donor of the antiserum. The glomerulo-nephritis produced in the rabbit by this means was most striking in its similarity to the human variety of the disease; indeed, so convincing were his illustrations that it was at once decided to attempt the repetition of his experiment as it was realised that if the method was reliable it constituted a weapon that had been eagerly sought after since the dawn of experimental medicine.
RABBIT KIDNEY EMULSION

INJECTED INTRAPERITONEALLY - DUCKS

DUCK SERUM

INJECTED INTRAVENOUSLY - RABBITS
Personal Experience of
Serum Nephritis.

Methods: Rabbits of varying breeds were
anaesthetised with ether and their abdomens opened. The renal artery on each side was cannulated by
means of a fine glass cannula, through which the organs were perfused with sterile saline until entirely free of blood. The kidneys were then removed and ground in a mortar. Normal saline was added in sufficient quantity to give a final suspension of 10 to 30 per cent. Aseptic precautions were observed throughout the preparation of the emulsion. Subsequently the suspension was injected, in 10 c.c. doses, intraperitoneally into Aylesbury ducks. This procedure was repeated at four to five-day intervals on twenty-five to forty occasions. At the end of this period each duck was anaesthetised and the sternum removed. With a wide-bore needle, as much blood as possible was aspirated from the right ventricle. The yield of blood varied from 60 to 110 c.c. Asepsis was/
Profusion of Rabbits' Kidneys.
"63" Magnification 125.

"63" Magnification 500.
was observed. The blood serum was heated to 56°C for thirty minutes in order to annihil natural complement and was then ready for use.

Results: A series of rabbits received two or three daily intravenous injections of varying doses of serum. The results were as follows:

Rabbit "63" received two successive doses of 5 and 3 c.c. serum. The animal was obviously ill and there was albuminuria, haematuria, and haemoglobinuria. Death occurred thirty-six hours after the first injection. At autopsy the kidneys were considerably enlarged and congested. The bladder contained dark-brown fluid. There was no other macroscopic abnormality.

Histology: There was a slight but definite increase in size of the individual cells of the glomeruli leading to some swelling of the tuft and a reduction in the patency of the capillaries. The convoluted tubules showed extensive cloudy swelling and hyaline droplet degeneration, while fatty degeneration was demonstrated by fat stains. In/
In the lumen of the tubules casts were present. The interstitium and vessels showed pronounced congestion.

Rabbit "66" received three successive doses of 5, 5, and 3 c.c. serum. It also showed obvious illness with albuminuria, haematuria, and haemoglobinuria. Death occurred eighty-four hours after the first injection. At autopsy the kidneys were found to be swollen to almost twice their normal size. They were dark red in colour. Other organs were normal.

Histology: The changes were in general similar to those already described in the case of the previous animal, except that they were more pronounced. The glomerular tufts showed very pronounced swelling both of the epithelial and endothelial cells and there was ischaemia of the capillary loops. There was, however, no definite increase in the number of nuclei in the glomeruli. The convoluted tubules showed very pronounced hyaline droplet degeneration and/
Magnification 95.

Magnification 75.

Magnification 290

Magnification 550.

Rabbit "67" Early.
and fatty degeneration. Beyond slight congestion the interstitium was healthy.

Rabbit "67" received two successive doses of 4 c.c. of serum. The animal did not appear to be greatly upset although there was moderate albuminuria and some haematuria. Six days after the first injection left nephrectomy was performed. At operation it was noted that both kidneys were macroscopically normal.

Histology of left kidney: The glomerular tufts were considerably increased in size; they were ischaemic, and there was a considerable increase in the number of nuclei in the tuft as well as definite swelling of the endothelial and epithelial cells of the tuft. So pronounced and characteristic were the glomerular changes in this kidney that one was convinced that one was reproducing a lesion very similar if not identical to that of human glomerulonephritis. The picture was strongly reminiscent of the illustration of the glomerular changes in Dunn and McNeely's (1917) famous description of "Trench Nephritis". There was some exudate into the much reduced/
Magnification 120.

Magnification 375.

Magnification 500.

Magnification 550.

Rabbit "67" Late.
reduced capsular space. The convoluted tubules showed advanced hyaline droplet and fatty degeneration. The interstitium was normal.

The animal continued to show albuminuria, lost weight, ate poorly and finally died twenty-one days after the first injection, i.e. fifteen days after the unilateral nephrectomy. At autopsy the remaining kidney was slightly larger than normal. The cortex was slightly broader and more pallid than usual. The other organs showed no obvious change.

**Histology of Right Kidney:** The glomeruli showed varying degrees of damage. Some were extensively fibrosed with obliteration of the glomerular space and with periglomerular fibrosis. Others less severely damaged showed well-formed "epithelial crescents" and increase in glomerular nuclei. The least damaged showed merely increase of nuclei, avascularity and a few adhesions between the two layers of Bowman's capsule. In brief, although all the glomeruli were damaged, the degree of damage varied widely. The tubules showed a varying degree of degeneration, in some areas so advanced as to show commencing fibrous tissue replacement. There was interstitial tissue increase around the most severely damaged/
Magnification 120.

Magnification 505.

Magnification 500.

Magnification 460.

Rabbit "68" Early.
damaged glomeruli. The vessels showed no obvious change.

Histologically the other organs showed no departure from the normal.

Rabbit "68" received two injections of 3 c.c. Although it showed no general disturbance there was albuminuria and slight haematuria. This animal had repeated observations of blood pressure carried out by the carotid loop method as described. The blood pressure showed a prompt elevation of approximately 30 mm. Hg., the pressure remaining up for thirty days. On the eighteenth day following the first injection left nephrectomy was carried out. At operation neither kidney presented any obvious abnormality. However, there was considerable clear peritoneal effusion which was, unfortunately, contaminated with blood before it could be secured for biochemical examination.

Histology of Left Kidney: The changes were almost identical with those shown by Rabbit "67" at twenty-one days, consisting of varying degrees of glomerular damage ranging from almost complete hyalinisation to merely a cellular increase; "epithelial crescents" were abundant and well-formed.
Magnification 85.

Magnification 120.

Magnification 500.

Rabbit "68" Late.
Tubular and interstitial changes were similar to "67".

This animal remained in good health for a further period of sixty-two days, i.e. a total of eighty days after the first injection. It was then killed. At autopsy the right kidney was increased to about one-and-a-half times its normal size, an enlargement which is entirely explicable on the basis of compensatory hypertrophy. It presented no other obvious macroscopic abnormality. The other organs were healthy.

**Histology of Right Kidney:** The changes were patchy in distribution. In some areas the glomeruli were replaced by a disc of comparatively acellular fibrous tissue and the attendant tubules had undergone fibrous tissue replacement. Other glomeruli showed "epithelial crescent" formation with the surrounding tubules in a state of degeneration. In the least damaged areas the glomeruli showed some thickening of the fibrous stroma of the tuft with slight nuclear increase, the surrounding tubules being comparatively normal. The vessels showed no obvious abnormality. The appearances were such as to suggest that the glomeruli which had been severely damaged by the serum had undergone fibrosis, with death/
death of the whole nephron and replacement by fibrous tissue, while the less severely damaged nephrons had at least partially recovered and were, judging from the good health of the animal, able to maintain renal function at a satisfactory level.

Histology of the Other Organs: Sections of the liver, heart, lung, spleen, suprarenal, voluntary muscle and vessels were normal.

Normal Serum Controls: The serum of a normal unimmunised duck was obtained in the manner already described, heated to 56°C, for thirty minutes and injected intravenously into three rabbits in doses comparable to those already used. The animals showed no general or urinary changes following the injections. Left nephrectomy was carried out successfully in each of the three rabbits at seven, fourteen, and twenty-one day intervals, and they were then killed successively at further intervals of seven days. Both macroscopically and microscopically the kidneys and other tissues were normal.

Discussion: The results show that a serum prepared in the above manner causes a glomerulonephritis similar histologically, in all essential features/
features, to human glomerulo-nephritis. The specificity of the serum is a matter of considerable importance; Masugi (1933) working with rats and rabbits claimed that such a serum caused specific damage to the vessels of the liver while it did not damage the kidney. As a control to the above experiments a group of ducks were subjected to a series of injections of emulsion of rabbit kidney rendered as blood free as possible. So far such a serum has not produced any very striking changes in the liver with the exception of a very pronounced degree of glycogenic infiltration such as has never been seen in any normal control animal. The kidney showed no clinical or histological evidence of damage with the use of liver serum, with the exception of an isolated observation made by a collaborator (Kellar, 1937) which showed very pronounced renal damage with the formation of several "epithelial crescents". The whole question of liver damage is being investigated and will be made the subject of future publication. Several estimations of the precipitin activity of the nephrotoxic serum were made, using rabbit kidney emulsion. There was found to be no correlation between precipitin activity and nephrotoxic/
nephrotoxic power. A recent paper by Smadel (1936) confirms this observation and he also confirmed that renal damage occasionally appears after injections of other organ preparations. He found, however, that nephrotoxic activity did not result from immunisation with erythrocytes or serum. It was also possible to remove the nephrotoxic activity of an anti-kidney serum by absorption with kidney cells or fat-free kidney tissue. His conclusion was that "nephrotoxin appears to be an antibody that is relatively organ specific in its affinities."

Pfeiffer (1910) claimed that antigenic differences could be detected between the proteins of liver, spleen, kidney, etc., but Pearce, Karsner and Eisenbrey (1911) failed to confirm Pfeiffer's claims. More recently, Fleischer (1920), (1921) seems to have demonstrated some slight difference in the antigenic activity of the body proteins. In considering the question of specificity in connection with the kidney it must be realised that an immune serum with very little or no tissue specificity might cause almost exclusively glomerular damage because the glomerular capillaries pass through their walls a larger quantity of diffusible substances/
substances than any other group of capillaries in the body. The one point of contact so far established between the mechanism of human glomerulo-nephritis and that of this experimental glomerulo-nephritis is the fact that the human disease seems to occur in those cases of streptococcal infection in which there is a vigorous antibody response resulting in an unusually intense antigen-antibody reaction while, in the experimental lesion, there is undoubtedly an intense antigen-antibody reaction occurring in the rabbit. This hypothesis, published a year ago, has since found striking confirmation in the observation by Kellet (1936) that the blood complement is greatly reduced in human cases of acute nephritis. He suggested that a similar reduction in complement might occur in the experimental nephritis produced by an anti-kidney serum and a few recent personal observations have tended to show that such is the case.

Sato (1934), Hemprich (1934), Weiss (1935) and Koranyi and Hamori (1936) have confirmed the production of renal damage by the serum method.
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Research into the Hypertension of Serum Nephritis.

A method of producing a satisfactory glomerulonephritis having been found research was then performed on the following four problems:

(1) A study of the hypertension that accompanied serum nephritis.

(2) The effect of previous renal denervation on the intensity of the renal lesion.

(3) The effect of previous renal denervation on the hypertension.

(4) The effect of renal denervation on an established hypertension.

(1) Hypertension in Serum Nephritis.

It was soon discovered that each batch of serum showed a different nephrotoxic activity, therefore the results of each serum used will be illustrated by a typical example. The hypertensive response has been observed in thirty animals.

Serum "A". Rabbit "68" received two successive daily doses of 3 c.c. of serum. Blood pressure rose after 24 hours, and the average pressure for the following 25 days was 139 per cent. The hypertension lasted 36 days. Albuminuria was present/
Simple Hypertension.
present at the end of 24 hours and rapidly became heavy. Unilateral nephrectomy 18 days after the giving of serum showed very extensive glomerular changes of the type already described. This operation did not influence the blood pressure. At 80 days the animal was killed and the remaining kidney showed many renal units to be completely destroyed and fibrosed. The whole histological picture, however, gave the impression that the pathological process had produced a certain degree of damage and had then ceased to advance.

Serum "B". Rabbit "75" received three successive daily doses of 3, 3 and 5 c.c. of serum. Blood pressure rose after 9 days. The average pressure for the following 22 days was 130 per cent. Albuminuria appeared 12 days after the first injection, and soon became heavy. The hypertension lasted 87 days at which time the animal was given a further course of serum which, unfortunately, produced that type of protracted anaphylactic shock which has been described by Coca (1927). There was a progressive fall in blood pressure and death occurred 10 days later. This later course of injections somewhat marred the original histological picture, but there was/
Simple Hypertension.

Simple Hypertension.
was definite scarring, indicative of the original lesion.

Serum "C". Rabbit "80" received three successive daily injections of 2, 3 and 4 c.c. of serum. The blood pressure rose after 10 days, and the average pressure for the following 20 days was 119 per cent. The hypertension lasted 70 days. Albuminuria appeared 4 days after the first injection and became moderate in intensity. This animal is still alive.

Serum "D". Rabbit "128" received three successive daily doses of 4 c.c. serum. Hypertension appeared in 5 days and the average pressure for the following 19 days was 120 per cent. The duration of hypertension was 27 days. Albuminuria appeared on the 8th day and became heavy. This animal is still alive.

To illustrate the occurrence of definite renal damage with a relatively slight hypertension the case of rabbit "126" is described. This animal received three successive daily injections of 5cc. of serum "D". The blood pressure rose in 2 days and the average pressure for the following 14 days was only 111 per cent. Histologically the glomerular tufts showed/
Simple Hypertension.

(Showing less response.)
showed very definite swelling and nuclear increase with several "epithelial crescents", a degree of renal damage found to be associated with a more pronounced hypertension in other animals.

**Serum "L".** Rabbit "5" received two successive daily doses of 5 c.c. serum. Hypertension appeared in 4 days, and the average pressure during the ensuing 19 days was 129 per cent. Albuminuria appeared on the third day, and became heavy. The animal is still alive and no histology available, but other observations show that this serum has never failed to produce a severe lesion when used in the above doses.

**Summary.**

The results indicate that serum nephritis is associated with a definite hypertension, the characteristics of which vary considerably when different batches of serum are used. The interval between the first injection and the onset of hypertension may be as short as 24 hours or as long as 10 days, and the duration of the hypertension ranges from a fortnight to a permanent elevation of pressure, although the usual period lies between 30 and 40 days. It has also been found that the intensity of the histological changes does not appear to parallel the/
the degree of hypertension; for instance, serum "B" produced a very pronounced hypertension with a degree of histological change not nearly so severe as in some examples of serum "D" in which hypertension was relatively slight. Albuminuria may precede or follow the onset of hypertension.

(2) The Effect of Previous Renal Denervation on the Intensity of the Renal Lesion.

Before proceeding to study the effect of renal denervation on the hypertension of serum nephritis it was obviously desirable to ascertain whether the denervation altered the intensity of the renal lesion. Müller, Petersen and Rieder (1930) reported some experiments in which they subjected dogs, with one kidney denervated, to heavy intravenous injections of B. coli followed in 30 minutes by chilling. Albumin, erythrocytes, and bacteria appeared promptly in the urine from the normally innervated kidney; whereas the urine from the denervated side continued to be normal. Milles, Müller and Petersen (1931) considered that renal denervation in the dog is followed by degenerative changes in the intima of the blood vessels.

In five rabbits denervation of the left kidney was/
Effect of Denervation on Intensity of Lesion.
Left Side Denervated. Right Side Intact.
was performed and they were then given three successive daily injections of 5 c.c. of serum. The animals were killed at intervals varying from 10 - 56 days. The histological changes in the two kidneys were compared, the intact organ serving as a control for the denervated kidney. In no case was there found to be any detectable variation in the intensity of the changes on the two sides. Illustrations of the normal and denervated kidneys - one early and the other late - of two of these animals are shown. The results, therefore, indicate that denervation does not alter the intensity of the nephritic process.

(3) The Effect of Previous Renal Denervation on the Hypertension.

Seven animals which had made a satisfactory recovery from the operation of left denervation and right nephrectomy were subjected, after a suitable interval, to a series of injections of serum of known hypertensive power. It has already been shown that the hypertensive characteristics of each serum vary. Therefore, in illustrating the results, those obtained from each of the three sera are grouped separately. For comparison, the/
Prevention of Hypertension.
the pressures of the control group of normally innervated animals are illustrated on the same chart. The pressures are calculated as percentages of the average normal pressure during the control period.

Group 1, Serum "B". The average pressure of the three denervated animals, after the injection of serum, was 98 per cent, whereas that of the control group was 130 per cent.

Group 2, Serum "C". The average pressure of the denervated animals, after injection of serum, was 101 per cent, whereas that of the control group was 119 per cent.

Group 3, Serum "L". The average pressure of the three denervated animals, after the injection of serum, was 100 per cent, whereas that of the control group was 129 per cent.

Summary.

Previous renal denervation prevents the occurrence of the hypertension of serum nephritis.

(4) Renal Denervation in Established Hypertension.

Having found that renal denervation prevented the occurrence of the hypertension of serum nephritis it was obviously desirable to study the effect of renal denervation on an established hyper-
tension/
Termination of Hypertension.

Termination of Hypertension.

Termination of Hypertension.
tension. It seemed likely that there would be considerable risk in carrying out the operation of right nephrectomy and left renal denervation in an animal suffering from acute serum nephritis. In order to minimise this risk the operation was performed in two stages. In four animals left renal denervation was done prior to giving serum and the right kidney removed when hypertension was well established. In one animal the reverse procedure was adopted, i.e. right nephrectomy was performed first and the left kidney denervated during the hypertensive phase. Several animals were rejected for the purpose of this experiment because the hypertension that followed the serum injections was not considered sufficiently adequate to demonstrate an unquestionable termination.

It will be seen from the five charts illustrated that the final severance of the renal nerves was followed by a dramatic fall in blood pressure. Reference to the charts illustrating the first section of this paper will show the type of hypertension which sera "D" and "L" produce in the normally innervated animal. It was noted that the termination of the hypertension did not lessen albuminuria/
Termination of Hypertension.

Termination of Hypertension.
albuminuria, and that there was no obvious change in the animals' general condition. That laparotomy per se does not lower the blood pressure is made abundantly clear by the fact that none of the operations carried out prior to giving serum altered the blood pressure, and reference to the chart of rabbit "68" will show that nephrectomy was carried out without effect upon blood pressure.

Summary.

Bilateral section of the renal nerve supply terminates the hypertension of serum nephritis.
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SUMMARY AND CONCLUSION.

The original results of this research may be summarised as follows:

(1) Hypertension is associated with oxalate nephritis, and is greater in degree when the animals are fed on a dry diet.

(2) Bilateral nephrectomy is not associated with hypertension.

(3) Injection of oxalate into bilaterally nephrectomised animals does not produce hypertension despite the fact that the period of survival is sufficiently long to permit of its appearance, if such were possible, in the absence of kidneys.

(4) Previous removal of all renal nervous connections prevents the occurrence of the hypertension of oxalate nephritis.

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(1) Glomerulo-nephritis results in rabbits from the injection of a nephrotoxic serum produced in the manner described.

(2) This glomerulo-nephritis is associated with hypertension.

(3) Previous denervation of a kidney does not alter the intensity of the glomerulo-nephritis in that organ.

(4)/
It will be realised from this equation that the dominant factor in the control of pressure in the arterial system is the calibre of the arterioles. Whereas pressure only varies directly as the first power of the minute volume of the heart or the coefficient of viscosity, it varies inversely as the fourth power of the radius of the arterioles. This formula only gives an approximation of the truth because it was devised for a system of rigid vessels. As yet the mathematics of a system of elastic vessels has defied the ingenuity of the physicist. However, one has the authority of Hess and Tigerstedt in stating that this formula furnishes a variable approximation which is at least of the correct order.

If then the arteriolar calibre be the dominant factor in blood pressure control, let us consider the factors which govern this calibre. In general these may be regarded as (1) nervous, (2) endocrine, and (3) toxic. The arteriolar bed is controlled by the efferent limb of an autonomic reflex arc. This arc is controlled by the vasomotor centre in the floor of the fourth ventricle. Depressor impulses from the carotid sinuses and the arch of the aorta travel in the afferent limb of this arc. The efferent fibres travel along the dorso-lumbar autonomic outflow.
Experimental interference with the efferent limb is productive of hypertension, as in denervation of the carotid sinuses and section of the aortic depressor nerve.

At present, so far as is known, pathological hypertension is not produced by defects in this autonomic arc. Curiously enough, there is apparently no exact information as to efferent pressor impulses operating on the vasomotor centre apart from the observation that powerful peripheral stimuli of many sorts may produce transitory elevation of pressure. The recent work of Braun and Samet in which they interrupted or prevented the experimental hypertension of depressor nerve section and of elevation of intracranial pressure by denervation of the kidneys rather suggests that powerful pressor impulses may originate in the kidneys. However, there is no evidence that mere denervation of the kidneys produces hypotension.

A review of the possible endocrine or toxic origin of hypertension has shown that so far there is no direct evidence of the activity of any endocrine factor in pathological hypertensive states, with the exception of basophil adenomata of the pituitary, the cortical tumours of the suprarenal and/
and medullary chromaffinomata.

Turning now to the more particular problem of renal hypertension, there is the teleological view that probably explains the necessity for hypertension in renal disease, although like all teleological explanations it affords no clue as to the mechanism at work. This view, originally advanced by Bier (1900), was to the effect that renal hypertension may be regarded as a compensatory mechanism brought into play to maintain glomerular filtration when there was a reduction in the number of functioning nephrons or a serious hindrance to the flow of blood through the glomeruli.

Assuming then, that renal hypertension is a compensatory mechanism, let us consider the means by which it is initiated and maintained. A simple and attractive hypothesis for which, unfortunately, there is little evidence is that hypertension is initiated by the retention in the body of some normally excreted urinary body. Some clue as to the possible origin of this hypertension may be afforded by considering experimental and clinical renal lesions associated with hypertension. The common pathological renal states characterised by hypertension are as follows:

(1)/
(1) Acute glomerulo-nephritis in which there is transient hypertension with a histological picture characterised by extensive occlusion of glomerular capillaries by endocapillaritis; (2) the late stages of glomerulo-nephritis in which there is extensive destruction of renal units with the surviving units extensively altered; (3) polycystic disease in which the state of affairs is essentially similar to that of a late glomerulo-nephritis, and (4) occasionally in prostatic hypertrophy with severe interruption of the passage of urine there is hypertension which disappears after operation.

The experimental renal lesions associated with hypertension are:

(1) Chronic reduction of the arterial supply to the kidneys is shown to cause hypertension (Goldblatt et alia, 1934); (2) chronic stasis of the kidney caused by partial obstruction to the renal vein causes chronic hypertension (Bell and Pederson, 1930-31); (3) blockage of the ureters, especially when associated with renal damage, has produced hypertension; (4) X-ray damage of the kidney which is associated with severe glomerular lesions has been observed to cause hypertension (Hartmann et alia, 1926); (5) the injection of nephrotoxic sera produces/
produces glomerular lesions similar to those in human glomerulo-nephritis and hypertension;
(6) transitory hypertension follows oxalate nephritis in which a tubular swelling causes a transitory glomerular ischaemia. It is possible that the common factor between all these varied clinical and experimental varieties of renal hypertension may be interference with the glomerular circulation. It is reasonable to assume that as filtration takes place in the glomeruli and that as an effective filtration pressure must be maintained there exist powerful mechanisms directed towards the maintenance of such a pressure. In the absence of any demonstrable blood-borne toxic or endocrine agent one is forced to conclude that the hypertension may be mediated by afferent autonomic impulses from the kidney to the central nervous system. Experimental proof of the existence of such a mechanism has been afforded by producing an experimental hypertension of renal origin and then by means of denervation of the kidneys preventing the development of the elevation of pressure.

While this research was in progress there appeared reports which shed considerable light on the problem. Pickering (1936a) and Prinzmetal and Wilson/
Wilson (1936) carried out independently a clinical research which led them to the conclusion that there was no evidence that excessive sympathetic tonus was responsible for the sustained hypertension found either in advanced renal disease or in essential hypertension. Pickering (1936 b) later in last year used the same methods to investigate a series of cases of acute glomerulo-nephritis, and the results led him to the conclusion that their hypertension was due to an excess of vaso-constrictor arteriolar tonus.

Page (1935) and Collins (1936) working with dogs have attempted to prevent the hypertension that results from the use of the Goldblatt clamp on the renal artery by previous renal denervation. They failed, and came to the conclusion that the renal nerves do not play a part in the chronic hypertension of renal ischaemia.

The results detailed in this thesis suggest that the more or less transient hypertension of acute renal damage can be prevented or terminated by renal denervation. These results appear to be at variance with those of Page and Collins, but there is a hypothesis that admits of both sets of observations. Certain personal clinical investigations/
investigations that are still in the initial stage have led one to realise more fully that arteriolar tonus is maintained by two factors, - (a) the autonomic constrictor impulses, and (b) the intrinsic tonus of the plain muscle of the arteriolar wall. Of these two factors the latter is far the most important, and it may well be that when the kidney is damaged and when there arises the necessity to raise the arterial blood pressure by a generalised arteriolar hypertonicity the first mechanism that operates is a rapid phasic nervous vasoconstriction, the operation of which can be prevented by renal denervation. Such a possibility is also indicated by Pickering's (1936 b) observations in acute nephritics. On the other hand if the renal damage is progressive and becomes chronic then a more sustained hypertension is mediated by an increase of the intrinsic tonus of the plain muscle of the arteriolar wall. Such is indépendent of nervous influence and hence is not susceptible to renal denervation (Page and Collins).

The results, therefore, described in this thesis are applicable only to the initial comparatively transient hypertension of acute renal damage/
damage. It is hoped in time to test the validity of this hypothesis by producing experimentally a chronic renal hypertension by means of a severe advanced glomerulo-nephritis, and by observing the effects of renal denervation in such a hypertension. Dogs are probably more suitable for the production of such a chronic hypertension, and at present goats are being subjected to a series of injections of dog kidney emulsion in order to obtain a serum which will produce the necessary glomerulo-nephritis.

The research described is to be regarded merely as the first stage in the investigation of renal hypertension. Given continued opportunity it is confidently anticipated that in the course of a few years additional light will be shed on the problem of renal hypertension.
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