OBSERVATIONS UPON THE MICROANATOMY OF THE IN VIVO QUICK-FROZEN AND DRIED KIDNEY IN NORMAL AND EXPERIMENTAL CONDITIONS

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ABSTRACT

OBSERVATIONS UPON THE MICROANATOMY OF THE
IN VIVO QUICK-FROZEN AND DRIED KIDNEY
IN NORMAL AND EXPERIMENTAL CONDITIONS

by William Charles Waggoner

Conventional histologic slides do not adequately portray living functional cellular anatomy of kidneys. Previous attempts to show living morphological details covered only normal conditions in three species. The present study was undertaken to expand earlier work and it is an attempt to visualize kidney anatomy more accurately than heretofore in normal and experimental conditions.

Kidneys of ten species were frozen, *in vivo*, cut, lyophilized, stained and compared to contralateral kidneys processed by conventional histologic methods. *In vivo* frozen-dried kidney sections averaged 34 percent intertubular area and 13 percent tubular luminal area and showed red blood cell rich blood vessels. Blood stasis, as indicated by red blood cell packing in capillaries, and proximal tubular luminal stenosis occurred when intrarenal pressure relations were altered with blood vessel, ureter, or lymph duct ligation. Also, lymph duct ligation increased

intertubular spaces by 45 percent. Renal capsule ablation removed kidney distension limitations, increased tubular luminal area, and caused tubular epithelium to stretch and become thin. Adrenalin, histamine, and glucose injections altered intrarenal pressure relations and changed glomerular filtration rates as shown by proximal tubular luminal closure or dilation. Hypertrophied kidneys possessed significantly enlarged tubular, glomerular, and capsular diameters, but no distinction was made between simple hypertrophy and hyperplasia. Renal hypertensive kidneys developed tubular luminal stenosis, decreased intracapsular space (Bowman's capsule), and intertubular space, and blood stasis in varying degrees, depending upon the method used for producing the hypertension. Four day adrenoprival animal kidney sections showed decreases in intertubular and proximal tubular luminal space. Draining post mortem kidneys from the severed hilar vessels before freezing produced ischemic sections with autolysis and decreased tubular luminal space. Sections from kidneys, which were drained, frozen, cut, thawed, dried, and then stained, appeared to be similar to conventional histologic preparations.

It is concluded that there are vast cellular differences between kidneys processed by conventional histologic methods

and the *in vivo* freeze-dry technique. Kidney parts appear quite elastic and renal overdistension is prevented, in part, by the renal capsule. Large tubular and intertubular spaces of frozen controls indicate large volumes of extracellular fluid. The observed interdigitating tubular cells would increase intercellular area and lend greater constructive support to the functioning organ. Ligation of renal lymph ducts, renal vein, or ureter may increase intrarenal pressure significantly to collapse smaller renal blood vessels and, in turn, increase resistance to renal blood flow. Intravenous adrenalin appears to have a two-fold effect on renal tubules: primary tubular closure and secondary tubular dilation. Intravenous histamine drops systemic blood pressure and causes tubule luminal obliteration. The tubules of a hypertrophying encapsulated kidney appear to merge into the interstitial area and encroach on smaller blood vessels, thus increasing resistance to blood flow. Decreased intertubular and tubular luminal space in four-day adrenoprival rats likely result from decreased plasma volume and blood flow.

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By

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This volume is respectfully dedicated
to my wife, Joanna

"There is no difference between structure and function; they are the two sides of the same coin. If structure does not tell us anything about function, it means we have not looked at it correctly."

A. Szent-Györgi, 1951

"We must now become literate enough to read function out of design, for anatomy is the theater in which physiology takes place."

C. Sherrington, 1950

INTRODUCTION

Histology, the study of normal tissues, may be entering a new era. With the modern, rapid advancement in technology, new methods of preparing animal tissues have emerged. The biologist must revise generally accepted thinking with respect to normal cellular construction of tissues. What we are accustomed to seeing is not necessarily correct, and adjustments and reconstruction of concepts may be necessary.

It was sometimes tempting from physiological data to assume various aspects of living organ architecture which could not be directly observed. However, showing the correct in vivo construction of many organs has not been done generally.

An organ of controversy with regard to natural morphological structure is the kidney. Ordinary paraffin sections do not adequately project the physiological picture. In this frame of reference, the present study was undertaken to improve and expand the earlier attempts to portray the probable living kidney microanatomy.

The primary intent of the present work is to show, in various animals, the functional cellular architecture of the kidney during normal and experimental conditions.

HISTORICAL SURVEY

Optics

Optical properties of curved surfaces were first described by Euclid (590 B.C.), Ptolemy (127–151 A.D.), and by Alhazan at the beginning of the 11th century. It is not surprising that spectacle-making and practical optical science emerged simultaneously. In 1590 the Janssen brothers combined two convex lenses with a tube and built the first compound microscope. Later, Leeuwenhoek improved lenses sufficiently for use in scientific investigation. Faber of Bamberg, a resident physician in service of Pope Urban VII, gave the name of "microscope" to this optical instrument.

In the 17th century Athanasius Kirchner was the first to employ the microscope systematically in the study of diseases, although he erred in many of his interpretations. Later, Robert Hooke modified the optical instrument of Kirchner with a sub-stage condenser. (Cited by DeRobertis, et al., 1948.)

The Cell

In the 17th century Robert Hooke used his penknife to shave thin slices of cork and examine them under the

microscope. He observed large pores in the material and called them "cells," a term used earlier by Roger Bacon. The cell theory, as an organized unit, actually originated with Schleiden and was later confirmed by Schwann. Later R. Virchow in Vienna applied this theory to pathology demonstrating that pathological processes take place in cells and tissues. (Cited by DeRobertis, et al., 1948.)

Max Verworn (1895) emphasized the role of the cell in physiology when he stated: "It is to the cell that the study of every bodily function sooner or later drives us. In the muscle cell lies the problem of the heart-beat and that of muscular contraction; in the gland-cell reside the causes of secretion; in the epithelial cell, in the white blood cell, lies the problem of the absorption of food, and the secrets of the mind are hidden in the ganglion cell. . . If then physiology is not to rest content with the mere extension of our knowledge regarding gross activities of the human body, if it would seek a real explanation of the fundamental phenomena of life, it can only attain its end through the study of cell-physiology."

Freeze-Drying

According to Mann (1902) preparation of tissues for microscopical research by drying is the oldest of all methods.

He relates that Leeuwenhoek prepared muscle samples by this method in 1720, carried them in his pocket, and cut sections from time to time for miscroscopic examination. The procedure used by Altmann (1890) was more refined than this and involved the maintenance of small portions of tissue *in vacuo* at -20°C., over sulfuric acid, in a desiccator. After several days tissues were embedded *in vacuo* in paraffin wax. Mann himself made use of Altmann's method, employing a mixture of solid CO_2 and alcohol to freeze whole animals; subsequently he broke off small pieces and dried them *in vacuo* at -30°C. . In 1915 Bayliss used a similar method, but dried his tissues at a slightly lower temperature. The method was of little practical use until Gersh (1932) produced the forerunner of all modern freeze-drying apparatus, and his lead was followed by a large number of investigators. Modifications of the original Gersh apparatus, or of the technique for using it, have been described by Scott (1933), Goodspeed and Uber (1934), Hoerr (1936), Scott and Williams (1936), Packer and Scott (1942), Gersh (1948), Wang and Grossman (1949), Mendelow and Hamilton (1950), Stowell (1951), Glick and Malstrom (1952), Treffenberg (1953), Jensen (1954), Arcadi and Tessar (1954), Moberger, Lindström and Andersson (1954),

Lacy and Blundell (1955), Burstone (1956), Caprino (1956), and Naidoo and Pratt (1956). The earlier types of apparatus and the theory and practice of freeze-drying have been described by Neumann (1952). An account of the freeze-drying technique in biological research has been given by Bell (1955), and Cabrini (1955) has reviewed the fundamental process principles. The physicalchemical protoplasm freezing and drying properties have been studied by Kulenkampff (1956), by Mamulina and Orlova (1955), and also by Hartlieb (1954).

Pearse (1961) states that the ability to obtain rapid tissue freezing rate depends on the thermal conductivity of the liquid used for the freezing bath and, if the pieces of tissue are large, it will also depend substantially on their own conductivity. Scott (1933), Hoerr (1936) and Simpson (1941a and b) have stressed the necessity for using a cooling liquid of high conductivity at a temperature as low as possible. In his earlier experiments Gersh used liquid air (-195^{o}C.) for his quenching bath and, although this had a sufficiently low temperature, its conductivity was low because a vaporized air layer around the specimen prevented sufficiently rapid transference of heat.

In order to overcome this effect Scott (1933) recommended the use of ethyl alcohol cooled to -100°C. in place of liquid air, but this method had the disadvantage that ethanol is solid at -115°C. and very viscous at higher temperatures in this region. Hoerr (1936) introduced the method of placing the tissues in crude isopentane cooled to about -165°C. with liquid nitrogen. Crude isopentane solidifies at -190°C. and the temperature at which it is beginning to become viscous is usually employed. Hoerr also used isopentane cooled to -120°C. Various other fluids have been employed for cooling. Emmel (1946) tried isopentane mixed with dry butane to lower the melting point of the former, and Bell (1952) proposed a mixture of propane (3 volumes) and isopentane (1 volume) or, alternatively, pure propane, which has a melting point at -185°C. Some authors, surprisingly, continued to use liquid air directly for quenching (Pease and Baker, 1949) and found no advantage in using isopentane. Simpson (1941a) confirmed the excellence of isopentane technique but maintained that even by this means the resultant freezing was uneven unless very small pieces of tissue were used. He described three zones in the tissue caused by quenching: an outer zone in

which ice crystal formation was minimal and preservation of structure excellent, an intermediate zone in which structural distortion by large crystals was particularly bad, and an inner zone of better but relatively indifferent preservation. Simpson believed that structural changes in the intermediate zone were caused, while it was still not solid, by pressure from the hard frozen outer zone. Bell (1952), on the other hand, considered it represented a zone in which solidification temperature was too high for rapid nucleation to occur, but crystal growth rate was high. In the innermost zone, according to this author, solidification temperature is too high for rapid nucleation but also too high for crystal growth rate to produce large artifacts of the intermediate zone.

According to Simpson (1941b) the best practice for tissues required for cytological examination is to regulate the size of the sample and the temperature of the quenching bath so as to obtain entirely outer zone preservation. No means exist for calculating these factors for different tissues and the method of trial and error must be employed. Bell (1952) conducted a series of experiments regarding the speed of quenching. By placing a thermocouple within a length of excised mouse intestine and

plunging the tissue into cooled isopentane, he found the time taken to reach equilibrium with the bath averaged 10 seconds. The blank for the thermocouple was 2 seconds. The critical temperature of water (-40°C.) was reached in 4 seconds. Parker, et al. (1962) did a similar set of experiments in mouse kidneys. With the tip of the thermocouple 1 mm. below the surface of the kidney, the temperature was found to fall from 37°C. to -10°C. in less than 0.1 sec. and to -100°C. in 4 sec. With the thermocouple placed in the middle of the kidney, some 3 mm. from the surface, the temperature drop was slower: to -10°C. in 4 sec. and to -100°C. in 8 sec.

Cryostat—Microtome

Freezing microtomes provided with means for cooling the knife blade with an additional CO_2 jet were introduced into histological technique by Schultz-Brauns (1931a and b, 1932). Commercial models have been available for several years and have been used by a number of workers (Wachstein and Meisel, 1953) to obtain semi-serial sections for histological use.

The first cryostat-microtome was developed in Denmark by Linderstrøm-Lang and Morgensen (1938) for their studies

in quantitive histochemistry in which alternate frozen sections were taken for chemical estimation and histology. In the original model cold was maintained by blocks of dry ice, and from this to provision of refrigerating coils was a simple step. The microtome was modified with a glass plate in front of the knife edge which effectively prevented section rolling or curling. No real attempt was made to apply the cold microtome technique thus developed by the Danish authors to histological methods in general. In 1951 Coons and his collaborators made a number of technical improvements in designing a new cryostat-microtome of 6 cubic feet capacity for working temperatures between -16°C. and -18°C.

Intrarenal Pressure Relationships

Brodie (1914) and Brodie and Mackenzie (1914) quick-fixed kidneys of normal and experimental animals in formalin and measured nephron diameters. Using Poiseuille's equation and tubule measurements of control dog kidney paraffin sections, he calculated the glomerular driving pressure required to propel filtered urine the nephron length as 72 mm. Hg for an estimated flow of 1 ml. per minute. On histological slides of cat kidneys following decapsulation he found a profound increase in intracapsular volume of Bowman's capsule and an

increase in tubule diameters, although the increase of the lumen diameter was greater than outside tubule diameter. In kidneys with ligated ureters, blood stasis was present in the capillary beds and tubular luminal diameters were increased slightly.

Brodie reasoned that the tough kidney capsule prevented overdistension and he observed that after decapsulation the kidney weight increased over controls by 20%. Renal vein ligation, with decapsulation, produced no greater weight increase than decapsulation alone. Ureter ligation increased kidney weight after 5 to 15 minutes. He also observed that a diuretic kidney had a 30% gain in weight over a "resting" kidney.

Generally, Brodie concluded most kidney parts are highly elastic and overdistension is prevented by general inextensibility of the connective tissue framework and, most of all, the capsule; and glomerular pressure is transmitted throughout the tubules, transversely and longitudinally.

In 1950 Montgomery et al. blindly inserted blunt hypodermic needles directly into the kidney cortex and measured pressures of 20-25 mm. Hg. Later, Gottschalk (1952) used a 100 micron pipette in the same fashion and measured pressures of 4 to 19 mm. Hg in 65 rats, guinea pigs, rabbits,

and cats, and 12 to 25 mm. Hg in 8 dogs. They both believed that they were measuring intrarenal interstitial pressure.

Swann et al. (1955, 1956a & b, 1958, and 1960) showed that fluid draining from kidneys after excision was different from blood. The hematocrit was one-half normal systemic values. Most of the fluid drained from the renal vein and very little from the artery or ureter. They believed draining blood had been diluted by renal interstitial fluid. With a series of formulas, he calculated what he believed was the interstitial volume of the living distended kidney. He considered interstitial fluid to be a mixture of systemic blood plasma and tubular resorbate. Overbaugh et al. (1957) used Swann's (1955) procedure and found species variation interstitial volume: (expressed as percent of total functional kidney weight) 18.6% in mice, 23.0% in rats, 20.7% in cats, and 32.2% in dogs. Swann et al. (1958) showed that distending interstitial fluid contained much protein, and concluded that it migrates easily from the vascular bed to the interstitial space and back. Pease (1955a), in electron microscope studies, showed that the kidney vascular bed is very porous at the capillary endothelium with holes 400-500 Angstroms wide. Along this same line, but with a different experimental approach, Collings and Swann (1958) infused dog kidneys

with radioactive blood albumin and hypothesized penetration of albumin into the interstitial compartment.

Gottschalk (1952) ligated renal arteries of rats and showed that, although renal artery blood pressure dropped markedly and urine flow stopped, interstitial pressure was maintained at control levels. He consistently found interstitial pressure greater than venous pressure, and when the renal vein is ligated for a period of time, interstitial pressure and venous pressure become equal. After ligating ureters, interstitial pressure increased and, in addition noted that ureteral ligation in rabbits produced a swollen and cyanotic kidney resembling an organ congested by increased venous pressure. He reasoned that renal venous blood pressure contributes, in part, to the values of tubular and interstitial pressures.

Winton (1937) found a drop in urine flow during a rise in venous pressure or a drop in arterial pressure. Later, Winton (1951), Hinshaw et al. (1959), and Hinshaw et al. (1961) found a decrease in renal blood flow with ureter ligation.

Using the studies of Sugarman et al. (1942), LeBrie and Mayerson (1960) found that lymph flow in the dog kidney

increased about five times during partial occlusion of the vena cava. Haddy et al. (1958) also showed an increase in lymph flow with an increase in venous pressure.

As shown by Winton (1956), Gottschalk (1952), and Gottschalk and Mylle (1956), renal tissue pressures may rise to high levels subsequent to an increase in tissue fluid, vascular volume, or tubular volume. Haddy et al. (1958) interprets this as a result of indistensibility of both the kidney stroma and renal capsule. They reason, therefore, that elevating venous pressure may increase renal vascular resistance, in part, because greater filling of veins raises tissue pressure, thus partially collapsing high resistance vessels.

In micropuncture studies in rats, Gottschalk and Mylle (1956) found a mean intratubular pressure of 13.5 mm. Hg. After uretral and renal vein ligation they found an increase in intratubular pressure. They also noted, occasionally, an abrupt obliteration of tubular lumina while inserting mircopipettes into the tubules of functioning rat kidneys. They report: Collapse* of all or a portion of the visible

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* In the sense of disappearance of the lumen (Gottschalk, 1962)

tubules sometimes occured after accidental trauma to the animal, i.e. hemorrhage or anoxia, and rarely without demonstrable cause. Collapsed tubules appeared white and opaque, and their lumina were more or less obliterated."

In 1930, Edwards examined, under magnification, in vivo frog kidney tubules and their means of handling dyes. He observed that when a glomerulus had little or no blood flow through it, the dye in the tubule became very concentrated. This dye was apparently stationary in the tubule. He says: " . . . a coagulable or precipitable exudate appears in them." Later, when he made these same kidneys diuretic, the glomeruli became active and the tubules dilated. Edwards states: "That this dilation is the result of fluid flow through the tubule is shown by observing the concentrated column of dye become separated from the tubule walls on all sides, and as the relatively clear fluid rushes past this static column of dye and the tubule wall becomes tinged, cuff like, around the dye, while the fluid next to the wall remains untinged. Finally, the dye is washed entirely from the lumen."

With excellent electron microscopy Pease (1955a & b) shows extraordinary folding or intussusception, with variations in tubular plasma membrane spacing, demonstrable

in the basal region of tubular cells, and shows tubular basement membrane surface area to the interstitium may be vastly increased. He has no doubts that the brush border represents a simple extension of apical cytoplasm. Further, he states: "The apical portion of the proximal tubule is very sensitive to osmotic variables and appears radically different after seemingly minor changes in fixation procedure. Terminal changes indicate that the apical end of these cells is very labile. This portion of the cell may thus be swollen to such an extent that the tubular lumina will be obliterated and the brush processes jammed together." With the electron microscope Herman and Hanssen (1962) did similar studies and showed the apical border of the proximal tubule is quite sensitive to post mortem changes.

Parker et al. (1962) fixed mouse kidneys in vivo by ultra-quick freezing. The frozen kidneys were sectioned on a cold microtome, dehydrated, and stained for microscopic examination. All tubular lumina were found to be widely open, with tubular walls thin and stretched out; and a large intertubular space was observed. They also reported that if fixation by freezing is delayed for 10-20 seconds, the tubules have become filled with an

amorphous eosinophilic staining substance and the lumen reduced to a mere slit or obliterated. Hanssen (1960) and Herman and Hanssen (1962) showed similar findings, and, furthermore, found that luminal obliteration can be prevented by making animals diuretic.

By cutting with scissors, photographs of *in vivo* frozen kidney slides and then weighing the respective parts, Parker *et al.* (1962) found sections to consist of 45% tubular cells, 15% lumina, and 43% intertubular space, excluding all glomeruli and large blood vessels from the measurement. Peritubular capillaries were included in intertubular space measurement. Babin *et al.* (1961) attempted to show post mortem maps (relative areas) in renal disease studies and found an increase in interstitial area with hypertension in contrast to Swann *et al.* (1959), who believe that kidney interstitial spaces diminish in hypertension.

Drugs

"In an experiment that has long been considered a classic in renal physiology, Richards and Plant (1922a & b) showed that when a rabbit kidney is perfused at a pressure such as to maintain a constant blood flow, adrenalin causes

a rise in the requisite perfusion pressure and, at the same time, an increase in kidney volume, an observation confirmed in eviscerated rabbits and dogs. A rise in perfusion pressure can only mean increased renal resistance, presumably because of vasoconstriction, while an increase in renal volume points to vasodilation. Richards and Plant interpreted this paradox by suggesting that adrenalin acts preferentially to constrict the efferent glomerular arterioles, thus causing enlargement of the glomeruli and the preglomerular vessels and swelling of the kidney. This phenomonen has often been confirmed by Smith (1939-40), and it has been widely accepted that preferential efferent arteriolar constriction, by raising the glomerular pressure, would maintain the filtration rate despite a reduction in flood flow." (Cited by Smith, 1951.)

Ahlquist et al. (1954) injected epinephrine as a single rapid intravenous slug and noted that the renal blood flow decreased acutely. The constriction, presumably at the afferent glomerular arterioles, quickly disappeared leaving the renal blood flow subject to the elevated arterial pressure. The flow changes were reflected in the urinary output. During the acute constrictor phase, the urinary output ceased. When the flow increased, due to the elevated

pressure, the urine output was increased above control levels. Although Smith (1951) presumed the increased kidney volume, accompanying adrenalin infusion, was due to the interstital compartment expansion, Swann et al. (1952a) found that renal interstitial pressure drops with pressor doses of adrenalin. Using in vivo dyes and histologic sections, Winton (1937) injected adrenalin, intravenously, and observed that the number of active renal glomeruli was decreased, however, Smith (1951) thought that the reduction in renal plasma flow induced by adrealin is not accompanied by closure of any glomeruli, as was shown by the glucose tubular reabsorption, which remained at constant maximum rates. Page and McCubbin (1953) also considered adrenalin a renal vasoconstrictor, while Goodman and Gilman (1958) state adrenalin may reduce renal blood flow by 50%.

In human patients Bjering (1937) observed that histamine reduced both the creatinine and urea clearances, but simultaneous reduction in mean arterial pressures made the renal effects difficult to interpret. In addition, Page and McCubbin (1953) considered histamine a renal vasodilator.

In histologic sections of duretic dog kidneys, which were quick-fixed in formalin, Brodie (1914) observed an increase in glomerular and Bowman's capsule diameters. Gottschalk and Mylle (1956) found that rat kidney intratubular pressures increased with intravenous hypertonic glucose injections. Bloom and Fawcett (1962), in histologic sections of formalin-fixed diuretic kidneys, found kidney tubular epithelium flattened with an extended brush border. Longley and Burstone (1962) believed that postmortem swelling of proximal tubules has an osmotic basis, as it is reduced or eliminated in kidneys perfused and fixed in hypertonic solutions.

Renal Hypertension

Hypertension syndrome is described back as far as 2600 B.C. in the Yellow Emperor's Classic of Internal Medicine as the illness associated with a hard pulse. In 1694 Giorgio Baglivi of Rome performed a necropsy on the body of the famous Italian physiologist and anatomist, Marcello Malphighi of Bologna, and indicated Malpighi's fatal apoplexy was due to cerebral hemorrhage secondary to renal hypertension. Stephen Hale's studies were by far the most significant contribution of the 18th

century toward hypertension research, when he reported experimental proof that flowing blood exerts a pressure on the walls of blood vessels. Although induration of the kidneys with oliguria, hematuria, and edema had been described by physicians for more than 1,000 years, it remained for Richard Bright, in 1827, to associate clinical findings of albuminuria, hardness and fullness of pulse, edema, and hypertrophy of the left ventricle with pathologic findings of sclerosing, contracted kidneys. (Cited by Skelton, 1962.)

In 1898 Tigerstedt and Bergman attempted to draw attention to the possible importance of a blood-pressure-raising substance formed in the kidney. Ruyter (1925) first described a peculiar type of kidney cell, which seemed to be in the afferent arteriole region of the glomerulus. It was left to Goormaghtigh (1939) to directly correlate hypergranulation of these juxtaglomerular cells with a degree of renal ischemia and hypertension.

In 1940 Page and Helmer and Braun-Menéndez *et al.* (1946) simultaneously extracted renin from kidneys of several species and it was regarded as a proteolytic enzyme which acts on the alpha-2-globulin of plasma to produce the pressor polypeptide, now known as angiotensin.

Soskin and Saphir (1932) readily produced renal hypertension by enclosing the kidney(s) in a collodion-gauze cast. Later, Goldblatt et al. (1934) initiated an avalanche of hypertension research by placing silver clamps on renal arteries, reducing renal blood flow, and causing sytemic hypertension.

With the Goldblatt kidney Mason et al. (1940) found renal arterial pressure dropped distal to the clamp which was placed on the renal artery. After hypertension ensued and blood pressure in the kidney returned to control states, systemic hypertension still remained. They also noted no decrease in oxygen consumption of experimental kidneys. Corcoran and Page (1941) noted that kidneys under chronic Goldblatt constriction showed no decrease in blood flow and could not, therefore, be correctly designated "ischemic."

Swann et al. (1952b, 1959) produced perinephric hypertension, after Soskin and Saphir, and found interstitial pressure values from 25 to 60 mm. Hg. However, the Goldblatt kidney showed no change in interstitial pressure. In the collodion-gauze encapsulated kidney Swann showed weight gain was as fast as hypertrophying kidneys and fluid volume draining from the wrapped kidney was greatly decreased. Therefore, he reasoned, the renal cells hypertrophied into intercellular spaces and decreased

pre-existing values of interstitium.

MacKay et al. (1938) and Moustgaard (1948) showed that hypertrophying kidneys increase in both wet and dry weight. Using tritiated thymidine and DNA assay, Miyada and Kurnick (1961) found increased incorporation of the former and increased content of the latter in rat kidneys following unilateral nephrectomy. These studies indicated both hyperplasia and hypertrophy play significant roles in compensatory renal growth. McCoy (1960) got similar results in control kidneys, but noted no dry weight gain in collodion and gauze-wrapped kidneys of hypertensive rats. He interpreted this as a mere hypertrophy with no hyperplasia in a wrapped kidney during hypertension.

Effect of Adrenalectomy

In 1962 Swann drained post mortem kidneys of rats, 4 days after total adrenalectomy, and showed the volume of blood in renal distending fluids to be 4/5 of controls. Also, the volume of fluid, which had been measured as interstitial fluid, shrank to 1/5 its normal value. These results were probably due to a decrease in plasma volume, body weight, and blood pressure (Turner, 1955).

PROCEDURE AND MATERIALS

Animals

Initially, kidneys of fish, frog, turtle, pigeon, mouse, rat, guinea pig, rabbit, cat, and dog were studied comparing tissues of conventional histologic procedures to those of the *in vivo* frozen-dried technique. Secondly, *in vivo* frozen-dried kidneys of experimental rats, cats, and dogs were studied. All animals were carefully screened and examined for disease. Dogs were negative for Leptospira pomona, icterohemorrhagiae, and canicola blood antibodies. In addition, dogs were given Leptospira canicola-icterohemorrhagiae and canine distemper vaccines (Fromm). At least three animals of each kind were used in all studies.

In Vivo, Freezing-Drying Technique

Animals were anesthetized according to Table 1 and placed in a supine position. A lengthy midline incision opened fully the peritoneal cavity. The kidneys were exposed freeing them from all adherent tissue except for the hilar vessels and the ureter. Not less than 50 ml. of 2-methylbutane (Eastman) were cooled with liquid air to -175°C. . Temperature measurements were made with a cryoscopic thermometer (Sargent - S 80135). The cooled isopentane was poured

TABLE 1

	ANIMAL	ANESTHETIC
1.	Fish (*Salmo gairdnerii*)*	Tricaine Methanesulphonate (MS222) (Sandoz) Swim in 1:20,000
2.	Frog (*Rana catesbeiana*)	Ethyl Carbamate (Merck) 5 mgm./gm. (dorsal lymph sac)
3.	Turtle (*Chrysemys picta*)	Double Pith
4.	Pigeon (*Columba livia*)	Diethyl Ether (Merck)
5.	Mouse (*Mus familiaris*)	Diethyl Ether
6.	Rat (*Rattus rattus*) Carworth Farms Nelson	Diethyl Ether - surgery Sodium Pentobarbital - blood pressure (Abbott) 30 mgm./Kg. Intraperitoneal
7.	Guinea Pig (*Cavia porcellus*)	Diethyl Ether
8.	Rabbit (*Oryctolagus cuniculus*)	Diethyl Ether
9.	Cat (*Felis catus*)	Diethyl Ether
10.	Dog (*Canis familiaris*)	Sodium Pentobarbital, 30 mgm./Kg. Intravenous, Surgery (Abbott) Sodium Pentothal, 20 mgm./Kg. Intravenous (hypnotic dose) blood pressure (Abbott)

* Classifications from Spector, 1956.

in a steady stream on the exposed renal surface. This freezes one side rapidly, but the side adjacent to the abdominal wall freezes slowly. Hence, only tissues from the exposed renal surface were prepared for microscopic study. The frozen kidney was grasped with cold tongs, cut loose with a cold surgical blade, and plunged into powdered dry ice.

Tissues were frozen to a chuck and cut at -15°C. and 8 microns on a Harris International Cryostat-Microtome (Model CT). Sections were placed individually on -15°C. coverglasses, seated in -15°C. miniature spacing racks, and placed in a Vertis lyophilizer (Model 10-102) for 18 hours at 20 microns of mercury. Following lyophilization tissues were stored in a dessicator until they were fixed to albuminized slides. Then, slides were flooded on a staining rack with alcoholic Wright's stain (Hartman-Leddon) for two minutes. Distilled water was added for one minute before the slide was washed thoroughly. Tissues were destained and cleared in ethanol, acetone, and xylene, respectively. Mounting from xylene was with Permount (Fisher) and glass coverslips.

Fig. 1. Freezing with Cooled Isopentane.

Isopentane (2-methylbutane) is poured into the large Pyrex test tube, which is then lowered into the Dewar flask containing liquid air. The cooling period, lasting about five minutes, brings the isopentane to -175°C. before it is poured over the functionally distended kidney.

Fig. 2. External View of Cryostat-Microtome.

Small thermostat button on the right side below rotary crank regulates cabinet temperature. Tissues are handled through the clear hinged lid. Apparatus is portable.

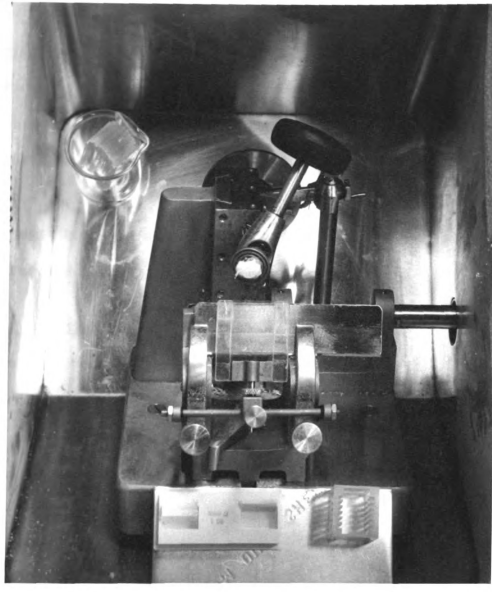


Fig. 3. Internal View of Cryostat-Microtome.

Sections slide off kidney between blade and anti-roll plate, and are placed on -15°C. coverslips, which are seated in the small plastic spacing rack (bottom right) for lyophilization. Present section thickness setting is at 8 microns (2 microns per division).

Conventional Histological Technique

Kidneys from freshly euthanized animals were fixed 24 hours in 10% formalin. After processing through the conventional solvents, tissues were blocked in paraffin, cut at 4 microns, placed on albuminized slides, and stained with hematoxylin and eosin. Mounting from xylene was with Permount and glass coverslips.

Vessel Ligation

In the rat the renal artery or vein was ligated for 30 seconds before kidney freezing. The ureter was ligated for 4 minutes previous to freezing. To augment lymph flow a dog was fed a pint of cream. The superior and inferior polar renal lymph ducts were ligated for 30 minutes prior to kidney freezing.

Renal Capsule Ablation

In the cat the renal artery was clamped and the organ became flaccid and shrank. The renal capsule was quickly stripped off and blood flow re-established for 5 minutes before kidney freezing.

Drugs

0.1 cc. Adrenalin chloride (Parke-Davis), 1:50,000, or 0.1 cc. histamine diphosphate (Abbott), 1:2,000, was injected via the vena cava in three rats, respectively. Thirty seconds ensued before kidney freezing. 0.5 cc. of 20% glucose was injected in three rats. Gross ureter peristalsis was observed before kidney freezing.

Hypertrophy and Renal Hypertension

Hypertrophy was obtained by unilateral nephrectomy and an ensuing period of not less than 21 days. Renal hypertension was induced by the Goldblatt (1934) method or variations of Soskin and Saphir (1932). These were: (A) Constriction of the left renal artery by a silver clamp and right renal ablation; (B) Collodion and gauze wrap of the left kidney and right renal ablation; (C) Collodion and gauze wrap of a hypertrophied kidney; or (D) Collodion and gauze wrap of the left kidney and right renal ablation after one week. Hypertension had been at least 7 days in duration at kidney freezing.

Adrenalectomy

Bilateral adrenalectomy was performed in three rats four days before kidney freezing.

Blood Pressure Measurements

Using the principle of Prioli and Winbury (1960) and Friebel and Vreden (1958) blood pressures were taken with the Physiograph (E & M) employing a cuff and pulse-sensitive transducer (see figures 4 & 5.). Systolic pressures were obtained from the dogs' hind feet and rats' tails. As shown in figure 5, a special warming box was used for rats.

Microphotography

All tissue photographs were with a Labolux (Leitz) microscope and Leica (Leitz) camera employing apochromatic (Zeiss) objective. Pantatomic-X and Kodachrome II Professional films (Eastman) recorded the slides.

Histological Slide Measurements

To show area percent occupied by kidney glomeruli, tubular cells, tubular lumina, and extracellular spaces, tracing paper was superimposed on kidney photographs and the respective structures were sketched. The sketches were cut out and the relative pieces weighed.

Direct diameter measurements were made on histological slides using a Microstar (A & O) binocular microscope employing a calibrated micrometer ocular.

Fig. 4. Blood Pressure Recording Apparatus.

Leads from the pressure cuff and pulse-sensitive transducer, coming from the rat blood pressure box, plug into the small electrosphygmograph base (upper left center). This instrument, integrating pulse wave and blood pressure recordings, plugs directly into a Physiograph amplifier. No special warming apparatus is necessary for dogs.

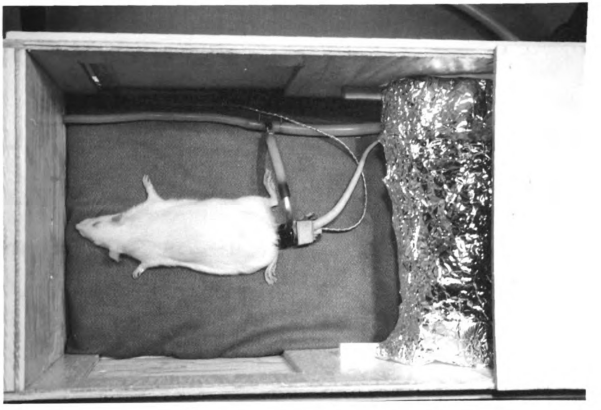


Fig. 5. Rat Blood Pressure Box.

The anesthetized rat (sodium pentobarbital) lies on a cushion with his tail through the cuff. A pulse transducer is attached to the tail's ventral side distal to the cuff. Two 40 watt light bulbs under a reflector (right) warm the tail and cause vasodilation.

In the anesthetized dog (sodium pentothal) the human style pressure cuff is wrapped on the hind leg and the pulse transducer is fitted on the ankle.

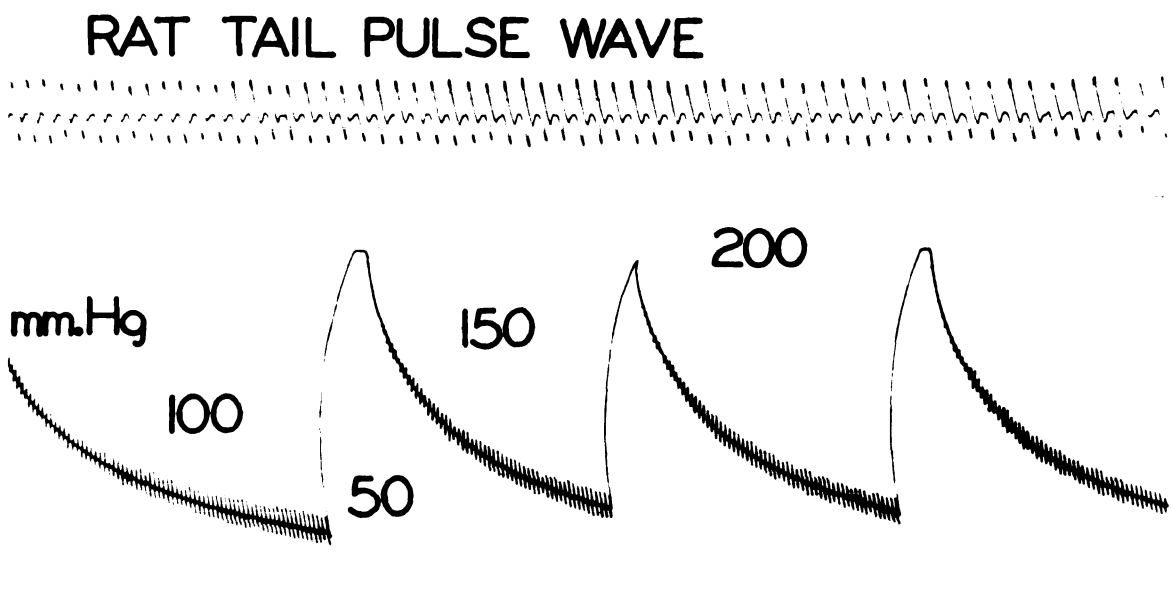


Fig. 6. Blood Pressure Records.

The top graph shows an amplified pulse wave from a rat tail using the pulse transducer and Physiograph. Note that the wave includes a dicrotic notch. Heart rate was 196/ minute.

On the same animal, the lower graph shows periodic cuff inflation and pressure decay. The pulse begins to make a strong appearance at 140 mm. Hg. Similar recordings are obtained with dog.

RESULTS

As seen in figures 7 through 27, the in vivo freeze-dry technique reveals microscopic details quite different from those conventionally pictured by histologists. In control kidney sections, all tubular lumina are widely open with thin epithelium. Large intertubular spaces are present containing red blood cell rich peritubular capillaries. Glomeruli, rich in red blood cells, project into Bowman's capsule leaving an intracapsular space. Figure 27 shows interdigitating cells found in the dog's thicker renal tubule walls. Effects of various experimental renal treatments are pictured in figures 28 through 42. Bloom and Fawcett (1962), Andrew (1959), and Edwards and Condorelli (1928) were used as references to morphological and microscopic detail for the various species studied.

Table 2 shows the percent of total renal cortical area or space devoted to glomeruli, tubular cells, lumina, and intertubular space, along with the number of tubular cross-sections used in each calculation. In this sort of kidney "map" study, peritubular capillaries were included as part of the intertubular compartment.

The symbols shown in Table 3 explain the column headings

in Tables 4 and 5. Table 4 is a listing of nephron diameters, in microns, of conventional histologic kidney slides in ten species. Table 5 is a listing of nephron diameters, in microns, of *in vivo* frozen-dried kidneys. For nephron diameter data, at least three structures on each of at least three different kidney slides were measured. In most cases ten structures of each classification were examined. Only those nephrons which were judged to be cross-sectioned at right angles to the long axis of the nephron were measured. Glomeruli, which were obviously sectioned acentrically, were omitted. Statistically, the sample means of the controls and experimentals were compared using the small sample method for fewer than 30 observations.

The graphs in figures 43 through 47 summarize the effects of renal treatments upon Bowman's capsule, glomerular, and proximal tubular luminal diameters.

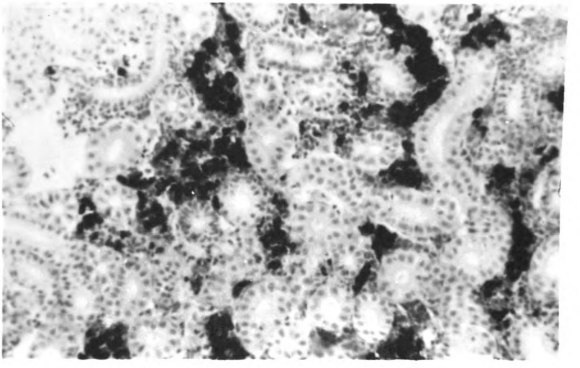


Fig. 7. Fish, Conventional Histologic Technique, H. & E., x300.

Fig. 8. Fish, In Vivo Freeze-Dry Technique, Wright's, x300.

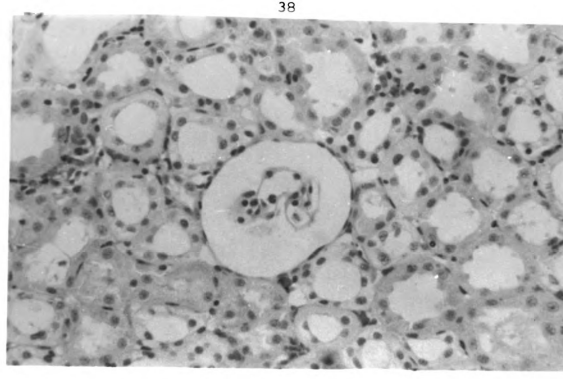


Fig. 9. Frog, Conventional Histologic Technique, H. & E., x300.

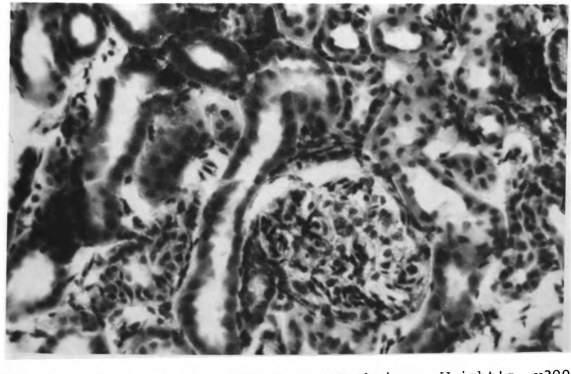


Fig. 10. Frog, In Vivo Freeze-Dry Technique, Wright's, x300.

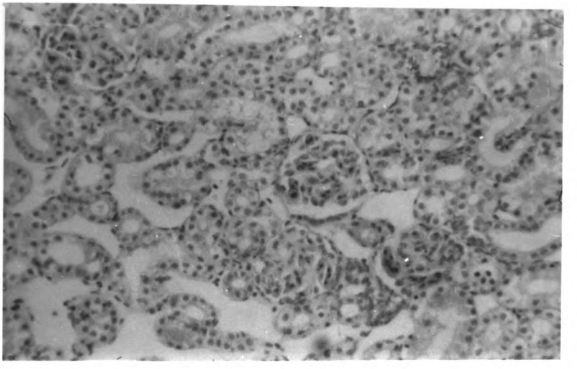


Fig. 11. Turtle, Conventional Histologic Technique, H. & E., x300.

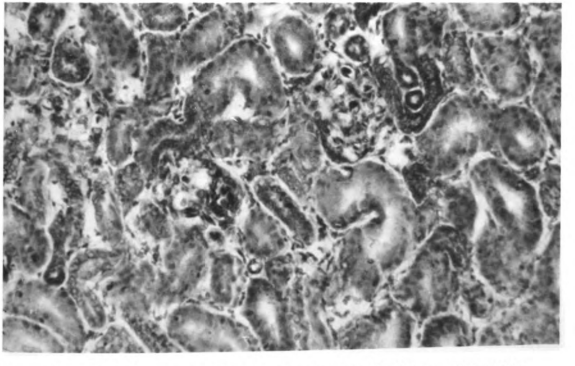


Fig. 12. Turtle, In Vivo Freeze-Dry Technique, Wright's, x300.

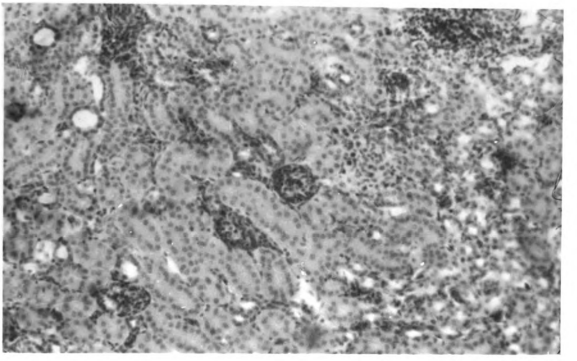


Fig. 13. Pigeon, Conventional Histologic Technique, H. & E., x300.

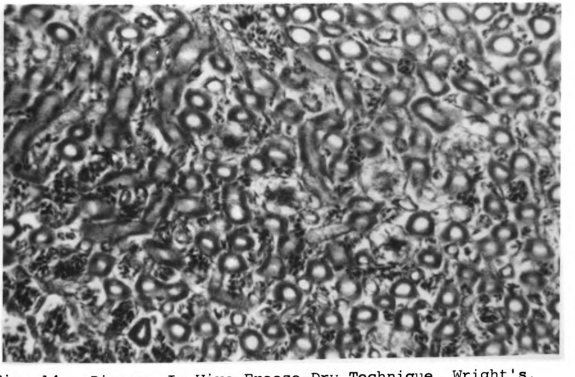


Fig. 14. Pigeon, In Vivo Freeze-Dry Technique, Wright's, x300.

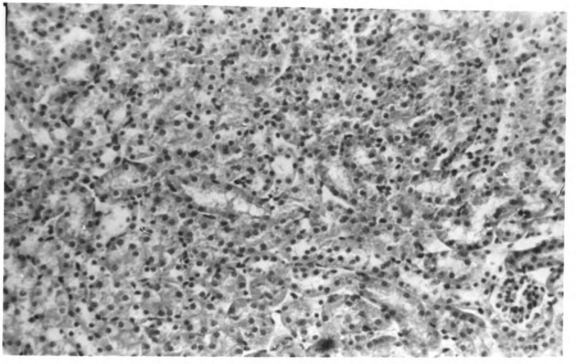


Fig. 15. Mouse, Conventional Histologic Technique, H. & E., x300.

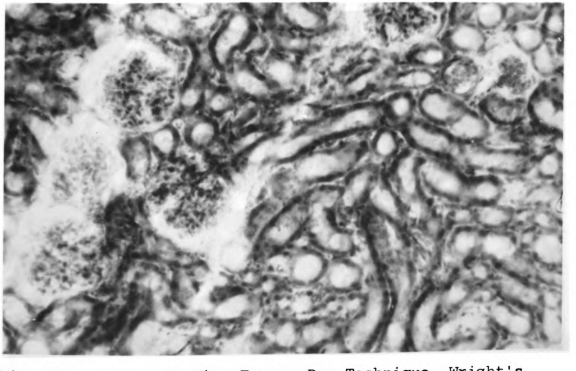


Fig. 16. Mouse, In Vivo Freeze-Dry Technique, Wright's, x300.

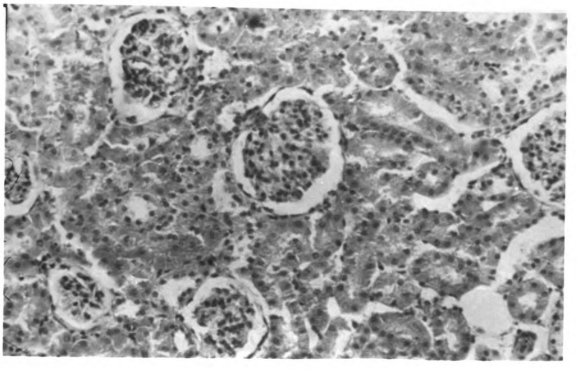


Fig. 17. Rat, Conventional Histologic Technique, H. & E., x300.

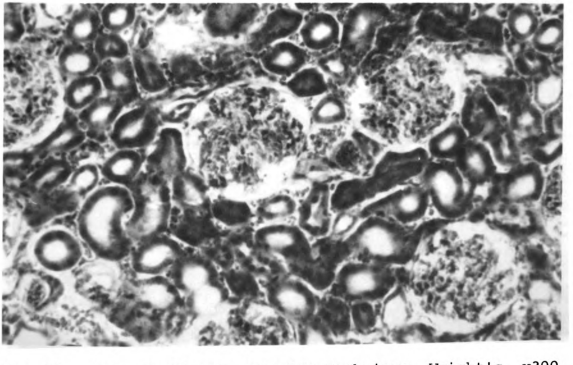


Fig. 18. Rat, In Vivo Freeze-Dry Technique, Wright's, x300.

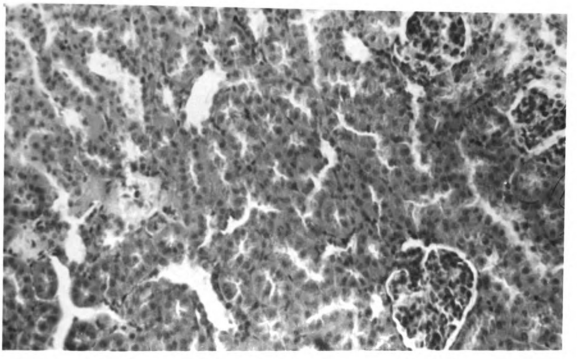


Fig. 19. Guinea Pig, Conventional Histologic Technique, H. & E., x300.

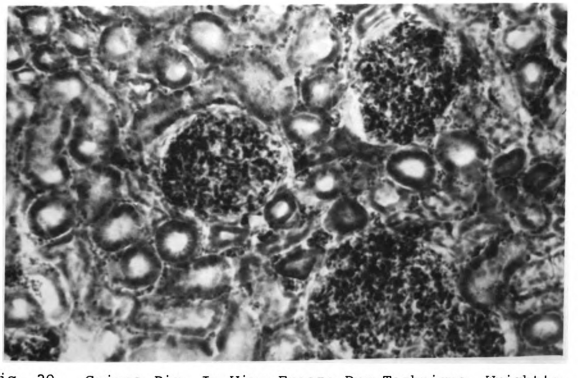


Fig. 20. Guinea Pig, In Vivo Freeze-Dry Technique, Wright's, x300.

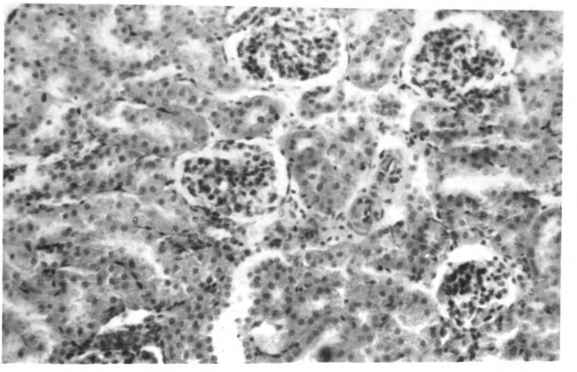


Fig. 21. Rabbit, Conventional Histologic Technique, H. & E., x300.

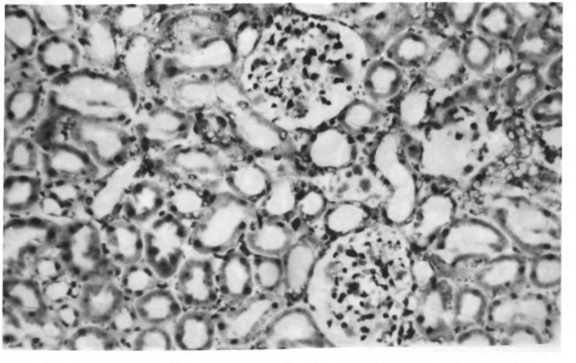


Fig. 22. Rabbit, In Vivo Freeze-Dry Technique, Wright's, x300.

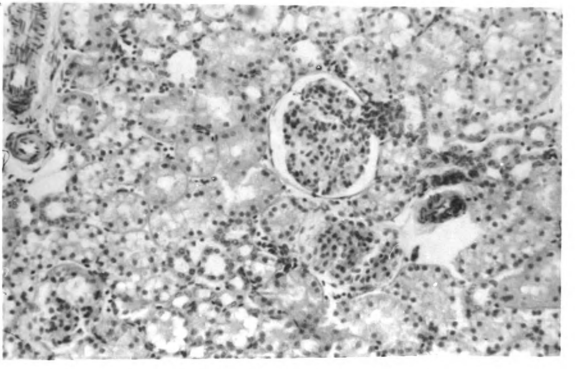


Fig. 23. Cat, Conventional Histologic Technique, H. & E., x300.

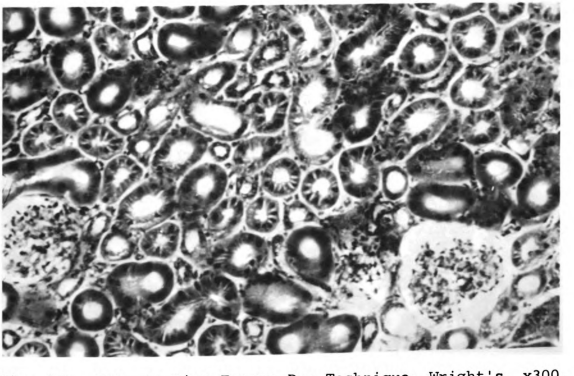


Fig. 24. Cat, In Vivo Freeze-Dry Technique, Wright's, x300.

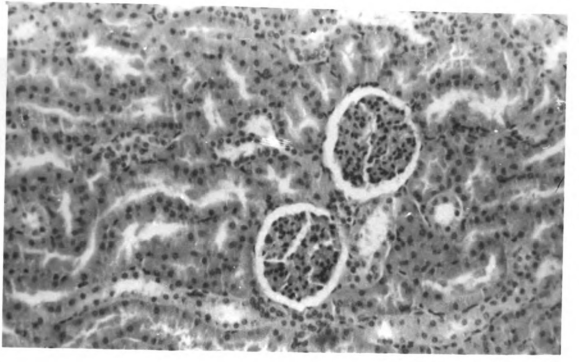


Fig. 25. Dog, Conventional Histologic Technique, H. & E., x300.

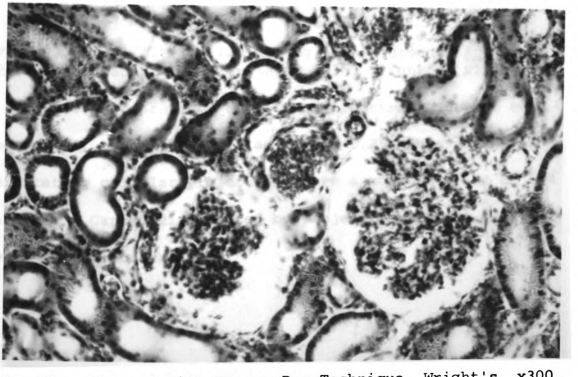


Fig. 26. Dog, In Vivo Freeze-Dry Technique, Wright's, x300.

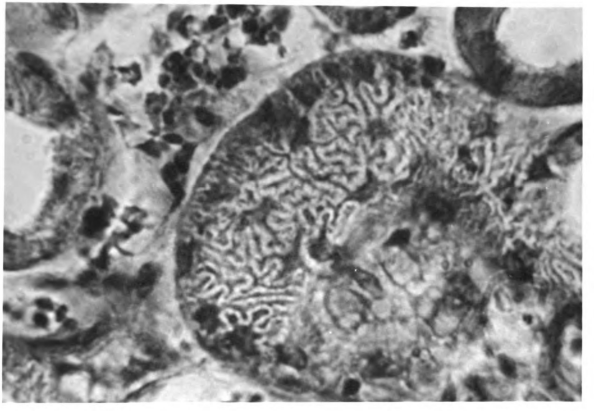


Fig. 27. Dog, B.P.* - 120 mm. Hg, No Treatment, In Vivo Freeze-Dry Technique, Proximal Tubule, Wright's, x2,000.

Nuclei appear to be stellate with interdigitating cell membranes. Mean nuclear diameter is 6.25 microns. Interdigitating cellular projections have a mean diameter of 1.4 microns. Canaliculi created by projections have a mean width of 2 microns.

* Blood pressure

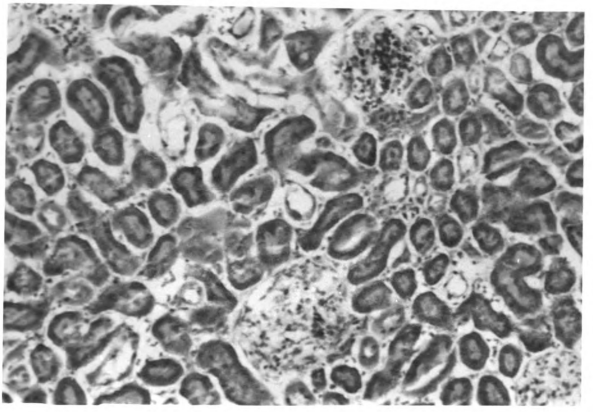


Fig. 28. Rat, B.P. - 117 mm. Hg, Renal Artery Ligation 15 sec. Before Kidney Freezing, In Vivo Freeze-Dry Technique, Wright's, x300.

The majority of tubule lumina are stenosed or obliterated with an eosinophilic staining substance. Space in Bowman's capsule is virtually absent. Several tubules, which appear to be distal, collecting, and Henle thin segments, remain open. Intertubular space is still abundant (see figure 18).

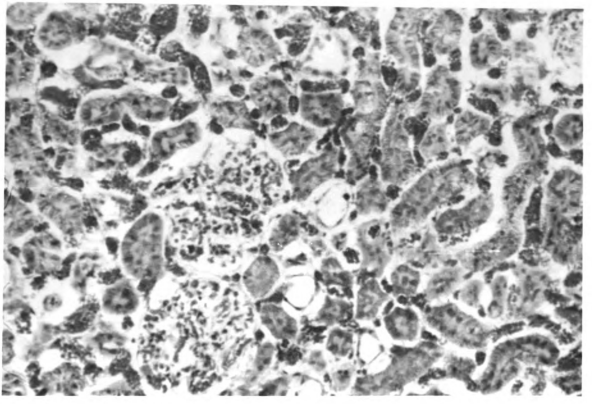


Fig. 29. Rat, B.P. - 130 mm. Hg, Renal Vein Ligation 30 sec. Before Kidney Freezing, In Vivo Freeze-Dry Technique, Wright's, x300.

Most of the tubule lumina are stenosed or obliterated, although the thin segments are more distended. Some space prevails in Bowman's capsule. Capillaries are engorged with red blood cells. Intertubular space appears to equal controls (see figure 18).

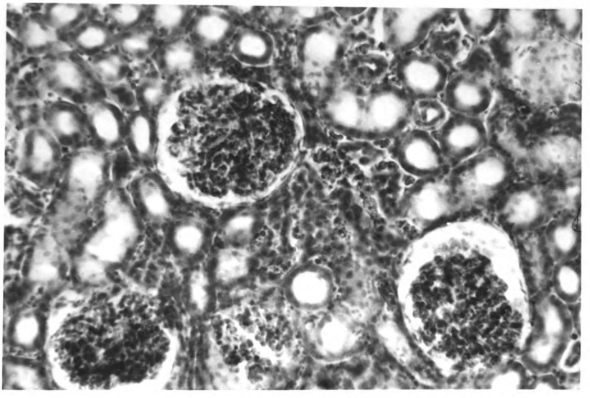


Fig. 30. Rat, B.P. - 105 mm. Hg, Ureter Ligation 4 Minutes Before Kidney Freezing, In Vivo Freeze-Dry Technique, Wright's, x300.

All tubules appear to be open and slightly more distended than controls (compare with figure 18). Capillaries contain more red cells and the intertubular space appears to be less than in controls.

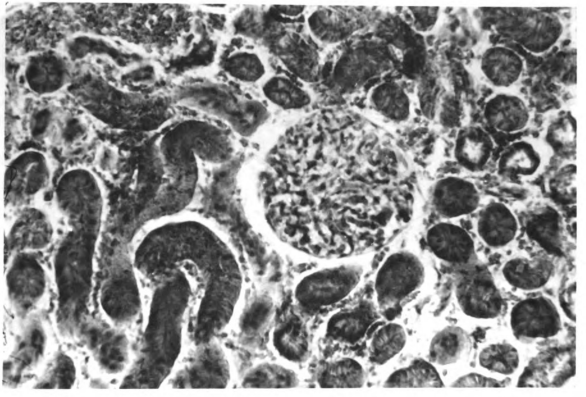


Fig. 31. Dog, B.P. - 130 mm. Hg, 30 min. After Ligation of Renal Lymph Ducts, In Vivo Freeze-Dry Technique, Wright's, x300.

This shows an increase in intertubular space. Most tubules are collapsed and space in Bowman's capsule is sparse. Compare with figure 26. Quantitatively, intertubular space here is 145% of controls.

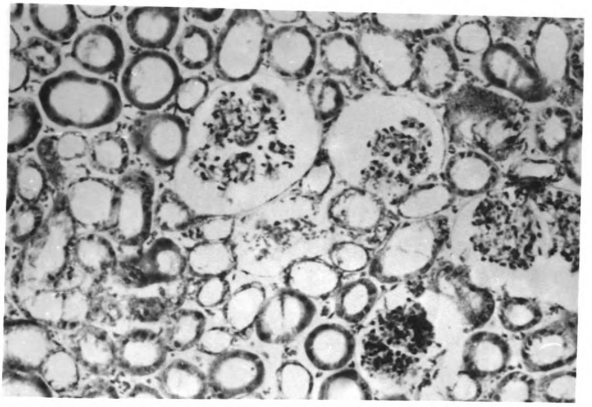


Fig. 32. Cat, Kidney Frozen After Renal Capsule Ablation, In Vivo Freeze-Dry Technique, Wright's, x300.

In comparison with figure 24, the tubules here have a greater inner and outer diameter with a thinner epithelium. Diameter of Bowman's capsules appears to be greater with no apparent change in the glomerular diameter.

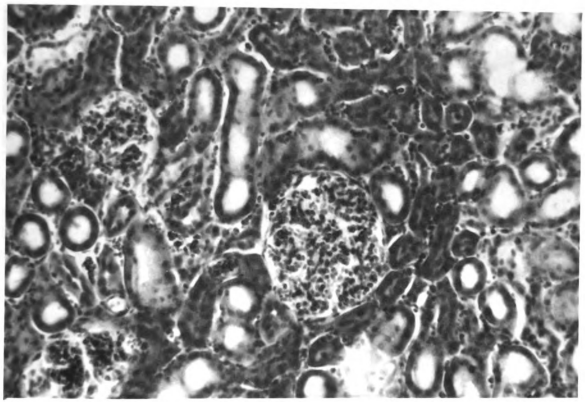


Fig. 33. Rat, Control B.P. - 95 mm. Hg, 0.1 cc. 1:50,000 Adrenalin Chloride I.V., In Vivo Freeze-Dry Technique, Wright's, x300.

Several tubule lumina are stenosed with the eosinophilic staining material. Space in Bowman's capsule is decreased. Comparing to figure 18, intertubular spaces appear to be about equal.

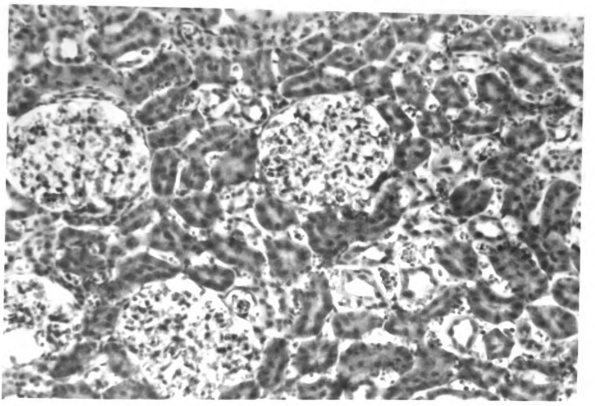


Fig. 34. Rat, Control B.P. - 112 mm. Hg, 0.1 cc. Histamine Diphosphate I.V., In Vivo Freeze-Dry Technique, Wright's, x300.

This appears similar to figure 28. The majority of tubule lumina are stenosed or obliterated with an eosinophilic staining material. Space in Bowman's capsule is sparse. Intertubular space is still abundant, although not as great as figure 28.

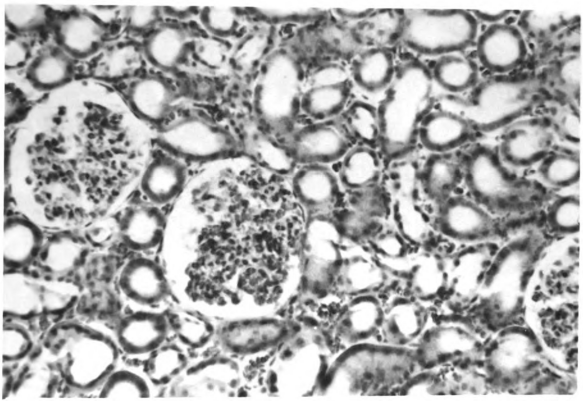


Fig. 35. Rat, Control B.P. - 127 mm. Hg, 0.5 cc. 20% glucose I.V., In Vivo Freeze-Dry Technique, Wright's, x300.

All spaces are clearly evident. Bowman's capsule and glomeruli appear larger than controls (see figure 18) and tubules have a greater inner and outer diameter.

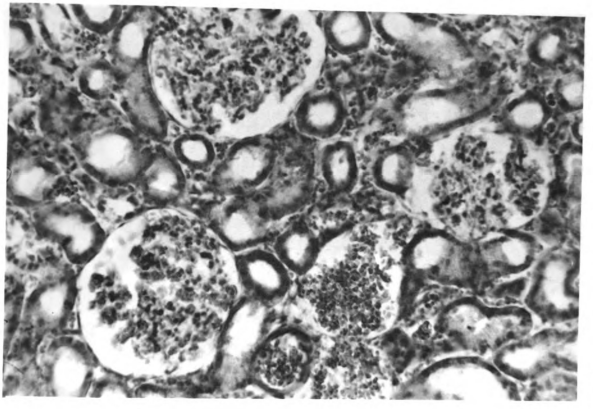


Fig. 36. Rat, Control B.P. - 140 mm. Hg, 21 day hypertrophy, In Vivo Freeze-Dry Technique, Wright's, x300.

Bowman's capsule, glomeruli, and inner and outer tubule diameters are larger than controls in figure 18.

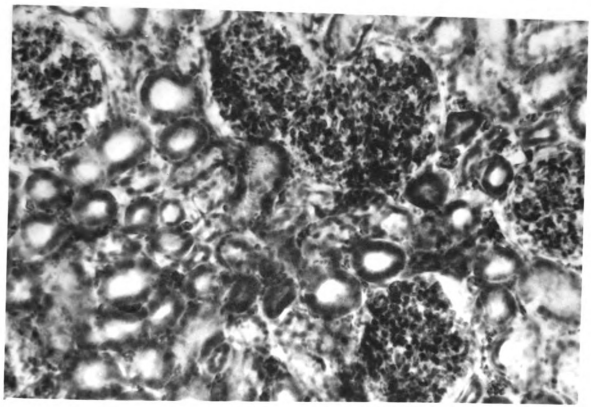


Fig. 37. Rat, Control B. P. - 112 mm. Hg, Silver Clamp on Renal Artery with Contralateral Nephrectomy, Hypertensive B.P. - 140 mm. Hg, In Vivo Freeze-Dry Technique at 7 days, Wright's, x300.

There is an apparent increase in Bowman's capsule, glomerular, and inner and outer tubular diameters. Some tubular lumina are obliterated. There is a decrease in intertubular and Bowman's capsular space with an increase of red blood cells in glomerular and peritubular capillaries.

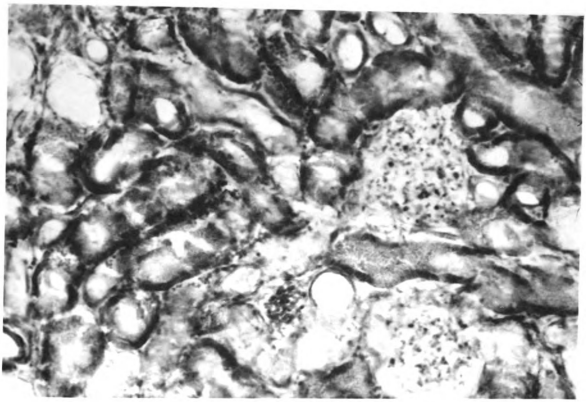


Fig. 38. Rat, Control B.P. - 95 mm. Hg, Collodion and Gauze Wrap and Contralateral Nephrectomy, Hypertensive B.P. - 178 mm. Hg, In Vivo Freeze-Dry Technique at 7 days, Wright's, x300.

There is marked necrosis and foreign body reaction. Eosinophilic staining is prevalent in the outer cortex. Alternating areas of blood stasis and ischemia appear throughout the section. Intertubular and Bowman's capsular space is greatly decreased.

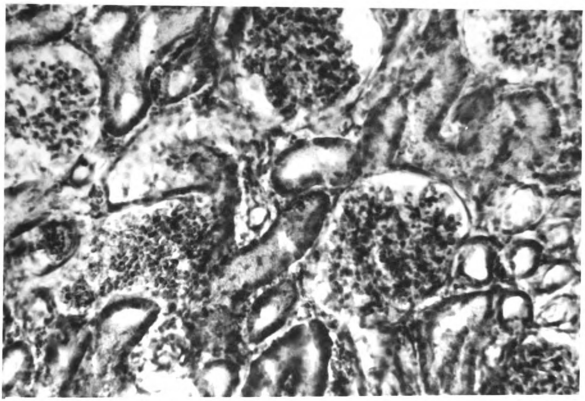


Fig. 39. Rat, Control B.P. - 110 mm. Hg, Collodion and Gauze Wrap of 21 Day Hypertrophied Kidney, Hypertensive B.P. - 158 mm. Hg, In Vivo Freeze-Dry Technique at 7 Days, Wright's, x300.

This shows little change from controls (see figure 36), although some tubular lumina are obliterated.

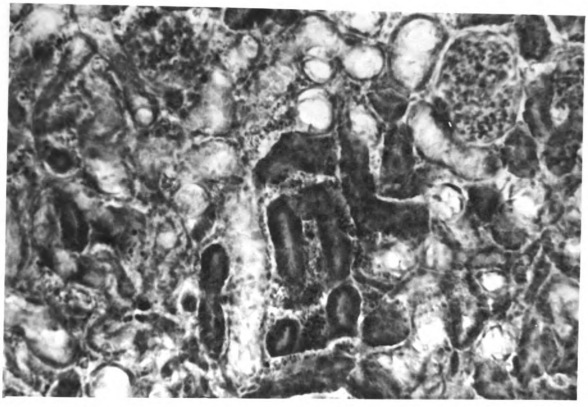


Fig. 40. Rat, Control B.P. - 112 mm. Hg, Collodion and Gauze Wrap Followed in 7 Days by Contralateral Nephrectomy, Hypertensive B.P. - 165 mm. Hg, In Vivo Freeze-Dry Technique at 7 Days, Wright's, x300.

Tubular lumina, which are not stenosed, appear to be distended greater than normal. As in figure 38, there are alternating areas of blood stasis and ischemia. Intertubular and Bowman's capsular space is minimal.

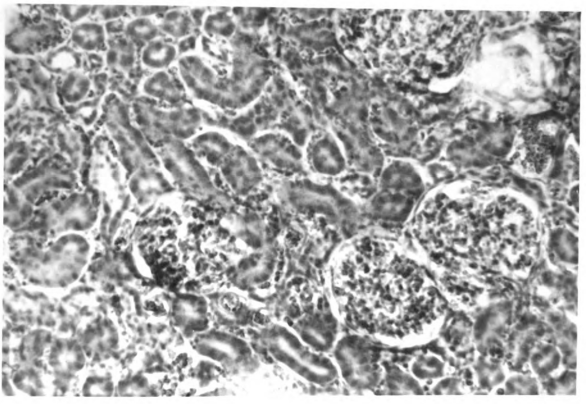


Fig. 41. Rat, Control B.P. - 125 mm. Hg, Bilateral Adrenalectomy, In Vivo Freeze-Dry Technique at 4 Days, Wright's, x300.

Many tubules are shrunken with obliterated lumina. There appears to be a small decrease in intertubular and Bowman's capsular space. This appears similar to figures 28 and 34.

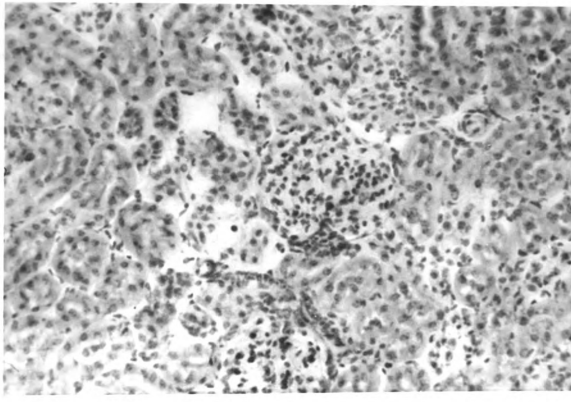


Fig. 42. Rat, Kidney Frozen After Excision, Cut Section Allowed to Melt on Warm Slide with No Lyophilization, Wright's, x300.

Appears similar to conventional histologic preparation, although there are no open tubule lumina and Bowman's capsular spaces. Contrast to figure 35.

TABLE 2

NUMBER OF TUBULAR CROSS-SECTIONS AND PERCENTILE AREAS OF RENAL CORTICAL SPACES

ANIMAL	NUMBER OF TUBULAR CROSS-SECTIONS	% GLOMERULI	% TUBULAR CELLS	% LUMINA	% INTER-TUBULAR SPACE
Fish	35	10	47	6	37
Frog	40	10	42	13	35
Turtle	78	8	49	10	33
Pigeon	178	11	37	8	44
Mouse	102	11	43	16	30
Rat	84	18	36	11	35
Guinea Pig	45	14	39	16	31
Rabbit	97	11	38	19	32
Cat	103	8	48	11	33
Dog	72	17	36	18	29

TABLE 3

SYMBOLS

ANIMAL - Animal used in experiment.

TREATMENT - Animal treatment before kidney freezing.

B.C.D. - Bowman's capsule mean diameter in microns.

G.D. - Glomerular tuft mean diameter in microns.

P.T.O.D. - Proximal tubule outside mean diameter in microns.

P.T.I.D. - Proximal tubule inside mean diameter in microns.

D.T.O.D. - Distal tubule outside mean diameter in microns.

D.T.I.D. - Distal tubule inside mean diameter in microns.

H.L.O.D. - Henle loop outside mean diameter in microns.

H.L.I.D. - Henle loop inside mean diameter in microns.

TABLE 4

NEPHRON DIAMETERS IN MICRONS FROM CONVENTIONAL KIDNEY SECTIONS

No.	ANIMAL	B.C.D.	G.D.
1.	Fish	45 ± 2.3*	37 ± 2.1
2.	Frog	142 ± 5.7	88 ± 4.2
3.	Turtle	115 ± 4.0	109 ± 4.1
4.	Pigeon	40 ± 1.9	40 ± 2.0
5.	Mouse	67 ± 2.0	61 ± 3.5
6.	Rat	112 ± 3.2	99 ± 4.1
7.	Guinea Pig	136 ± 3.7	120 ± 3.5
8.	Rabbit	109 ± 4.3	96 ± 3.7
9.	Cat	95 ± 2.1	83 ± 2.0
10.	Dog	120 ± 3.7	98 ± 3.6

NO.	P.T.O.D.	P.T.I.D.	D.T.O.D.
1.	45 ± 2.8	12 ± 1.5	- -
2.	74 ± 4.0	48 ± 3.6	64 ± 2.3
3.	64 ± 2.7	16 ± 1.2	40 ± 2.0
4.	36 ± 2.9	1 ± 0.8	24 ± 2.7
5.	40 ± 4.7	0 ± 0	40 ± 3.4
6.	40 ± 2.9	3 ± 0.8	38 ± 3.1
7.	35 ± 2.5	8 ± 1.2	43 ± 3.9
8.	35 ± 2.4	5 ± 2.1	40 ± 2.8
9.	40 ± 2.2	13 ± 4.2	29 ± 3.3
10.	39 ± 3.0	17 ± 1.6	32 ± 5.1

* Standard deviation

TABLE 4 (cont.)

NEPHRON DIAMETERS IN MICRONS FROM CONVENTIONAL KIDNEY SECTIONS

ANIMAL NO.	D.T.I.D.	H.L.O.D.	H.L.I.D.
1.	- -	- -	- -
2.	40 ± 3.6*	24 ± 2.2	14 ± 1.6
3.	17 ± 1.1	21 ± 3.9	6 ± 1.1
4.	8 ± 2.1	8 ± 1.3	6 ± 1.5
5.	16 ± 3.4	24 ± 2.7	19 ± 2.4
6.	16 ± 2.9	24 ± 2.0	16 ± 1.4
7.	29 ± 3.2	27 ± 3.0	16 ± 2.2
8.	16 ± 3.1	32 ± 2.0	16 ± 2.8
9.	16 ± 2.8	22 ± 2.5	13 ± 2.3
10.	16 ± 1.8	22 ± 1.9	12 ± 2.4

* Standard deviation

TABLE 5

NEPHRON DIAMETERS IN MICRONS OF IN VIVO FROZEN-DRIED KIDNEYS

NO.	ANIMAL	TREATMENT	B.C.D.
1.	Fish	None	56 ± 1.4*
2.	Frog	None	193 ± 5.9
3.	Turtle	None	135 ± 5.7
4.	Pigeon	None	47 ± 2.3
5.	Mouse	None	82 ± 5.4
6.	Rat	None	126 ± 4.1
7.	Guinea Pig	None	149 ± 4.9
8.	Rabbit	None	126 ± 6.3
9.	Cat	None	140 ± 7.5
10.	Dog	None	151 ± 8.1
11.	Rat	15 sec. renal artery ligation	107 ± 3.2
12.	Rat	30 sec. renal vein ligation	126 ± 3.0
13.	Rat	4 minute ureter ligation	118 ± 3.2
14.	Dog	30 minute lymph duct ligation	126 ± 5.9
15.	Cat	Renal capsule ablation	133 ± 7.7
16.	Rat	0.1 cc., 1:50,000 Adrenalin, I.V.	126 ± 5.9
17.	Rat	0.1 cc., 1:2,000 histamine, I.V.	110 ± 4.5
18.	Rat	0.5 cc., 20% glucose, I.V.	134 ± 4.1
19.	Rat	3 week hypertrophy	145 ± 9.2
20.	Rat	Hypertension (method "A")	118 ± 5.4
21.	Rat	Hypertension (method "B")	110 ± 4.9
22.	Rat	Hypertension (method "C")	141 ± 8.9
23.	Rat	Hypertension (method "D")	110 ± 5.7
24.	Rat	4 day adrenalectomy	102 ± 5.1
25.	Rat	Remove kidney and drain	118 ± 4.3
26.	Rat	Standard histochemical preparation	110 ± 3.9

* Standard deviation

TABLE 5 (cont.)

NEPHRON DIAMETERS IN MICRONS OF IN VIVO FROZEN-DRIED KIDNEYS

ANIMAL NO.	G.D.	P.T.O.D.	P.T.I.D.	D.T.O.D.
1.	56 ± 2.6	47 ± 3.7	14 ± 2.2	- -
2.	180 ± 5.1	71 ± 3.1	39 ± 3.3	71 ± 4.3
3.	125 ± 5.4	60 ± 4.5	15 ± 2.9	55 ± 3.7
4.	39 ± 1.7	28 ± 3.1	13 ± 1.4	24 ± 3.2
5.	68 ± 4.5	25 ± 3.4	13 ± 2.3	30 ± 3.2
6.	110 ± 6.7	31 ± 4.6	14 ± 3.4	35 ± 3.9
7.	118 ± 6.2	44 ± 5.1	27 ± 2.3	49 ± 4.1
8.	110 ± 5.2	41 ± 6.4	24 ± 3.4	47 ± 4.8
9.	118 ± 5.3	47 ± 5.8	23 ± 2.4	39 ± 5.0
10.	126 ± 4.3	39 ± 6.5	20 ± 2.2	39 ± 4.5
11.	107 ± 3.2	31 ± 4.1	0 -	33 ± 3.6
12.	118 ± 3.6	35 ± 3.2	0 -	47 ± 4.9
13.	110 ± 3.8	39 ± 4.8	15 ± 2.3	39 ± 4.2
14.	126 ± 5.9	35 ± 4.7	0 -	33 ± 2.7
15.	97 ± 8.0	44 ± 5.4	31 ± 3.4	47 ± 4.4
16.	110 ± 7.2	39 ± 6.1	24* ± 7.8	31 ± 2.9
17.	110 ± 4.5	31 ± 4.5	0 -	31 ± 3.6
18.	113 ± 7.1	42 ± 5.7	28 ± 4.4	38 ± 2.9
19.	126 ± 9.3	47 ± 7.3	31* ± 6.2	42 ± 5.3
20.	110 ± 5.6	39 ± 4.7	27* ± 7.2	50 ± 3.4
21.	102 ± 5.0	31 ± 3.6	22* ± 6.4	44 ± 6.6
22.	125 ± 8.4	39 ± 6.5	24* ± 6.5	31 ± 5.1
23.	94 ± 6.1	42 ± 4.3	25 ± 3.4	39 ± 4.4
24.	97 ± 4.4	36 ± 3.7	18* ± 2.1	19 ± 1.8
25.	118 ± 4.3	39 ± 3.5	0 -	30 ± 2.8
26.	110 ± 3.9	47 ± 3.6	0 -	31 ± 2.6

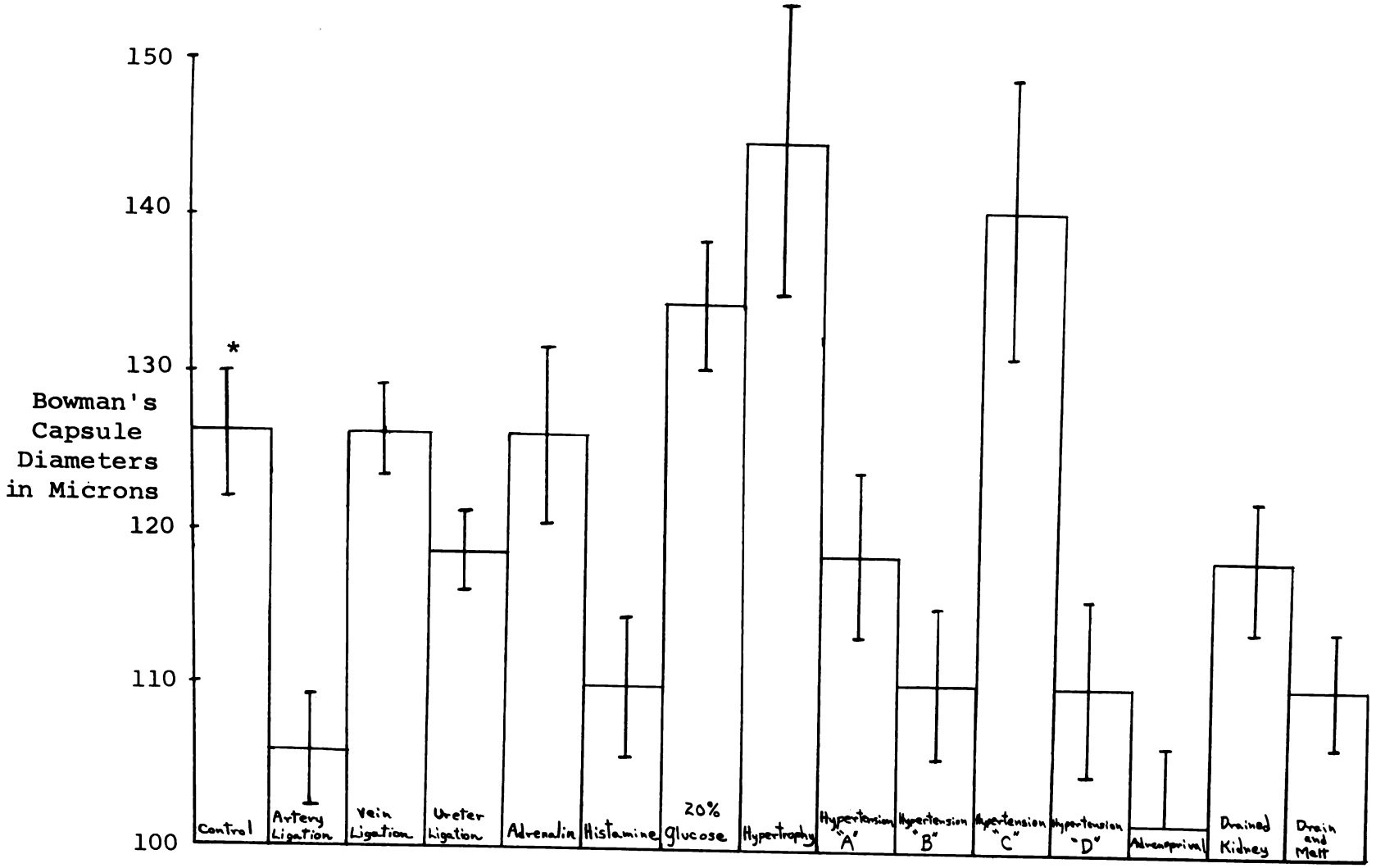
* Mean inside diameter of tubules remaining open

TABLE 5 (cont.)

NEPHRON DIAMETERS IN MICRONS OF IN VIVO FROZEN-DRIED KIDNEYS

ANIMAL NO.	D.T.I.D.	H.L.O.D.	H.L.I.D.
1.	- -	- -	- -
2.	39 ± 3.1*	42 ± 4.2	31 ± 3.8
3.	8 ± 1.7	19 ± 2.3	8 ± 2.1
4.	17 ± 2.6	13 ± 2.1	9 ± 2.8
5.	14 ± 2.5	22 ± 2.2	13 ± 2.0
6.	17 ± 2.8	23 ± 2.4	19 ± 1.9
7.	28 ± 3.3	24 ± 2.3	15 ± 2.2
8.	34 ± 4.2	20 ± 3.5	14 ± 2.9
9.	20 ± 3.1	23 ± 3.4	16 ± 1.7
10.	20 ± 3.4	27 ± 2.1	17 ± 2.3
11.	16 ± 3.8	19 ± 2.9	13 ± 2.5
12.	27 ± 4.0	31 ± 3.2	27 ± 2.8
13.	22 ± 3.5	31 ± 3.3	14 ± 2.3
14.	16 ± 2.4	14 ± 2.6	6 ± 2.2
15.	31 ± 3.0	24 ± 3.4	19 ± 3.2
16.	16 ± 2.3	24 ± 2.5	9 ± 1.7
17.	16 ± 3.3	19 ± 1.6	11 ± 1.3
18.	31 ± 2.4	24 ± 2.1	16 ± 1.2
19.	20 ± 3.6	36 ± 4.1	24 ± 3.4
20.	28 ± 3.3	31 ± 3.2	20 ± 2.7
21.	30 ± 5.3	30 ± 3.8	20 ± 2.9
22.	22 ± 4.6	35 ± 3.7	25 ± 3.3
23.	24 ± 4.2	24 ± 2.3	19 ± 2.5
24.	10 ± 1.6	27 ± 1.8	19 ± 1.2
25.	0 -	23 ± 2.4	16 ± 2.4
26.	0 -	19 ± 1.7	6 ± 1.6

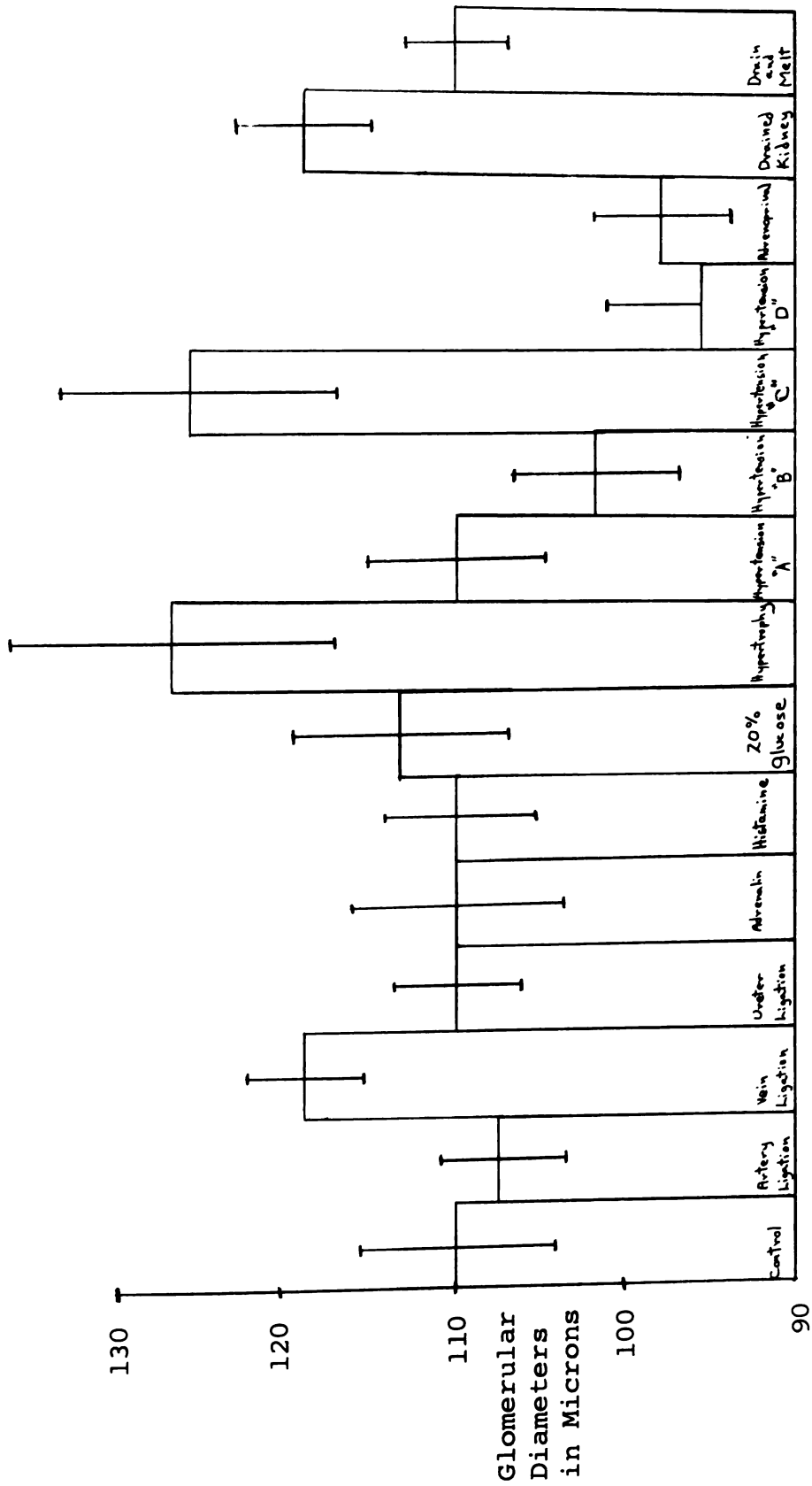
* Standard deviation



Experimental Treatment of Rat Kidneys

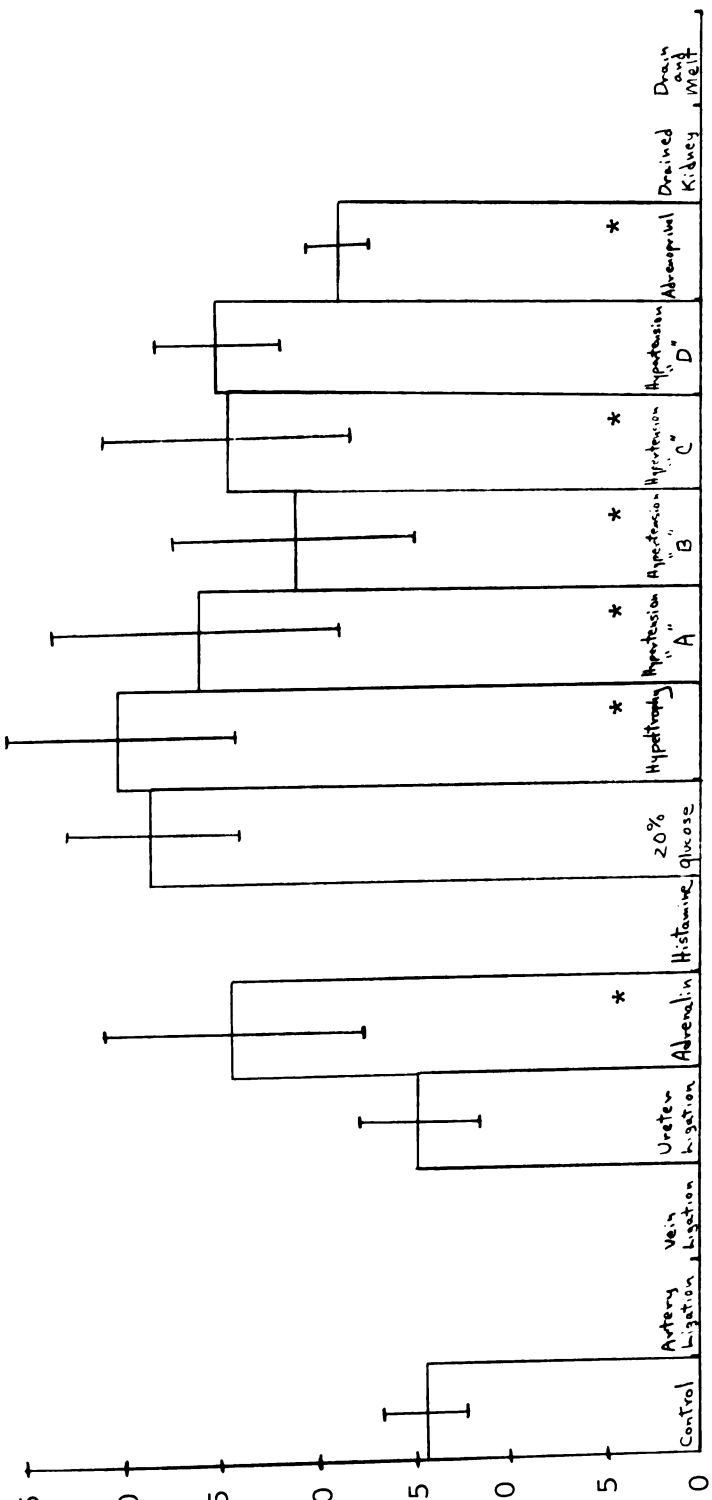
Fig. 43

* Standard deviation



Experimental Treatment of Rat Kidneys

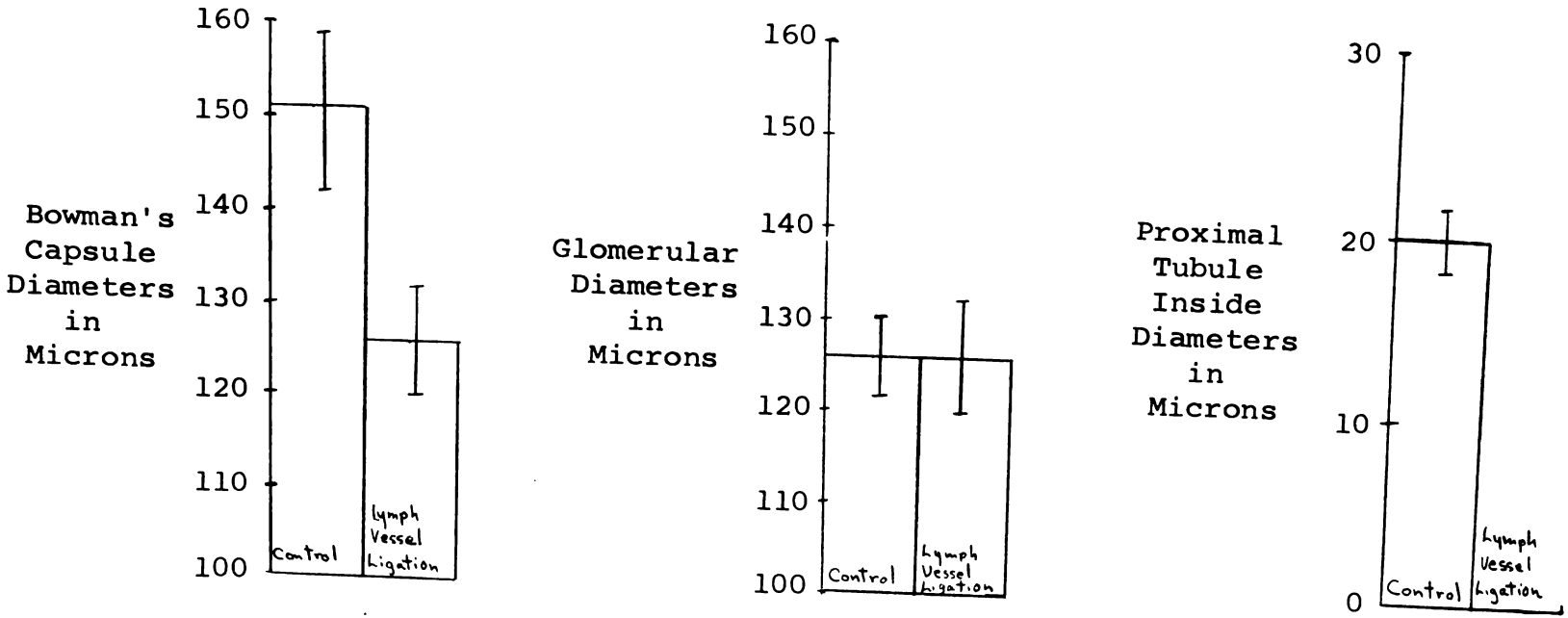
Fig. 44



Experimental Treatment of Rat Kidneys

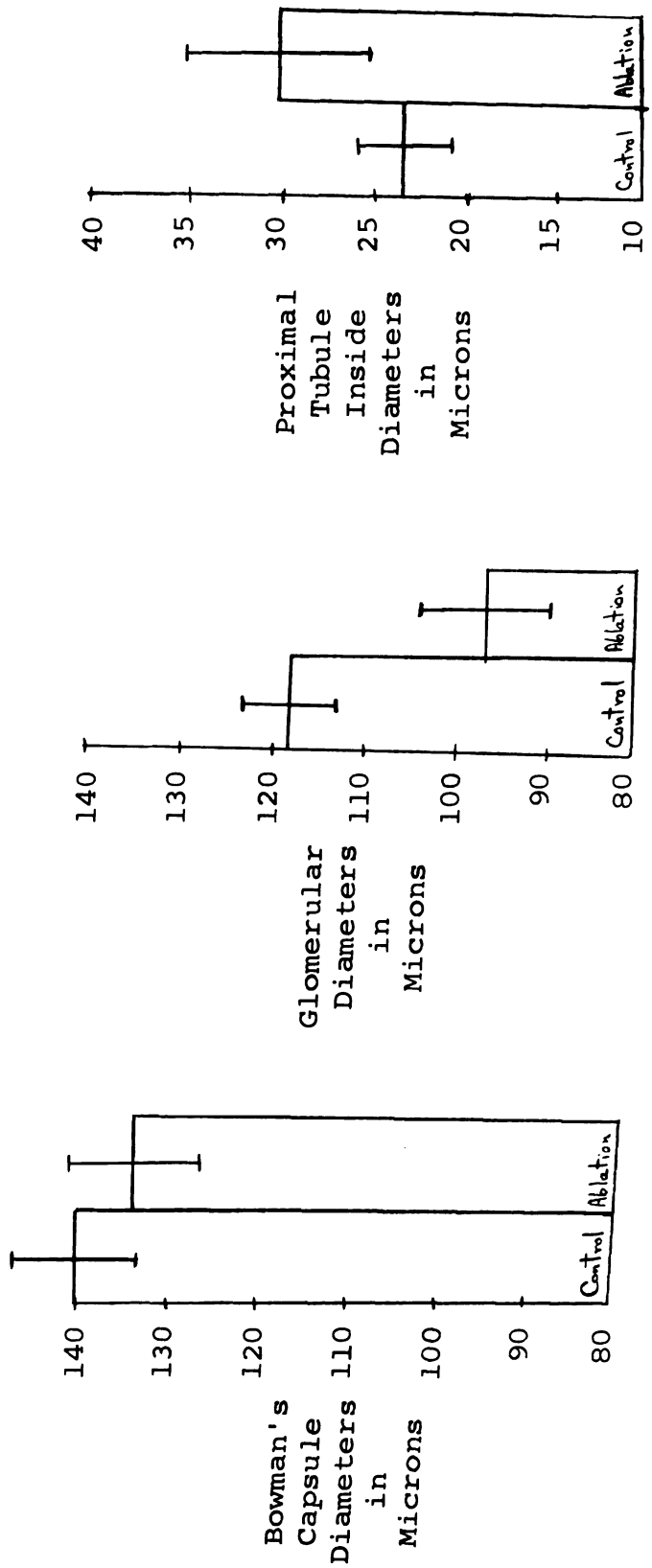
Fig. 45

* Mean inside diameter of tubules remaining open



Effect of Lymph Vessel Ligation in Dog Kidneys

Fig. 46



Effect of Renal Decapsulation in Cat Kidneys

Fig. 47

DISCUSSION AND CONCLUSIONS

Although it has limitations and could be improved, the *in vivo* freeze-drying technique offers scientific investigators a tool for a better insight to cellular anatomy and function. Some of the limitations to be considered are: the cryostat-microtome gives a variable tissue section thickness at any one setting; *in vivo* frozen-dried kidney sections show red blood cell shrinkage and intercellular membrane separation, thus there is some evidence for distortion; cellular and nuclear membrane tearing may result from handling of dried tissues; and Wright's stain shows different dye absorption characteristics by cells in sections from various species despite the same procedure and timing. Among the advantages of the technique are: prevention of post mortem diffusion and osmosis often encountered with other techniques; elimination of cellular autolysis; and nearly instant arrest of cells in the living state thus preserving the functional anatomical relations.

In the comparative study, as shown in figures 7 through 26, larger tubular luminal and intertubular spaces are present in the *in vivo* frozen-dried kidney sections (figures 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26) than in sections

from conventional histologic methods (figures 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25). The differences are smaller in fish, frog, and turtle, which have lower mean arterial pressures than in other species studied. Kidney sections by conventional methods are red cell poor because of post mortem drainage prior to fixation. Swann et al. (1955, 1956 a & b, 1958 a & b, 1960, 1962) have extensively reported on the fluid loss from the freshly excised kidney. Nephron diameters of in vivo frozen-dried kidney sections are 1 to 51 microns larger than sections from the conventional histologic methods. With conventional histologic methods tubular luminal diameters and loss of interstitial space could result from the fixation procedure and osmotic swelling.

The interdigitating nature of tubular cells with in vivo freeze-drying, as shown in figure 27, could be a result of freezing or lyophilization. Definite cellular spacing leads one to believe that considerable shrinkage or selective expansion occurred. Then too, this might be natural tubular cell conformation between apical and basement membranes. If the cell membranes do intususscept or interdigitate, as shown in figure 27 and by Pease (1955b & c), this would be an advantageous arrangement leading to increased intercellular surface area.

Using Brodie's (1941) form of Poiseuille's equation for fluid flow (pressure = $1.611 \times 10^4 \cdot L/r^4$ mm. Hg) and *in vivo* froze -dried kidney section tubular measurements, the minimum pressure required to force filtered urine through the nephron length is 9.33 mm. Hg in dogs and 11.91 mm. Hg in rats for a flow of 1 ml. per minute. This coincides fairly well with the 13.5 mm. Hg mean tubular pressure of rats as directly measured by Gottschalk and Mylle (1956).

Rat renal artery ligation 15 sec. before freezing (figure 28) produces a virtual obliteration of proximal tubular lumina and intracapsular space of Bowman's capsule. In addition there is a significant* decrease in the following diameters: Bowman's capsule (figure 43), glomerulus (figure 44), inside diameter of the proximal tubules (figure 45), and inside and outside diameters of Henle's loop (Table 5). Even though the proximal tubular inside diameter drops to zero (Table 5, figure 45), the outside diameter shows no significant difference from controls, and intertubular space remains abundant. Apparently, sufficient glomerular hydrostatic pressure and/or glomerular blood flow are necessary

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* Unless stated otherwise, significance in this discussion is at the 1% level as determined by the t distribution.

for maintaining capsule distance from the glomerular tuft. Blood pressure and flow are probably also necessary to maintain proximal tubular lumina. Proximal tubular luminal obliteration, as seen here, may be the overextension of the brush border as suggested by Pease (1955a & b).

Rat renal vein ligation 30 sec. before fixation by freezing (figure 29) produced marked red cell packing in peritubular and glomerular capillaries. No significant difference is shown with Bowman's capsule diameter (figure 43), although intracapsular space is diminished by significant glomerular expansion (figure 44). As with renal arterial ligation, tubular lumina are stenosed and intertubular space is abundant. Here, glomerular blood pressure is probably higher because of venous occlusion. However, glomerular blood flow is likely diminished and, as a result, filtration is minimal and tubular closure ensued, probably due to stasis of filtrate and its osmotic passage into the tubule cells.

Four minute rat ureter ligation (figure 30) increased significantly outside tubular and glomerular diameters and produced red cell packing in glomeruli and peritubular capillaries. This could have occurred as follows: ureter ligation raises ureteral resistance to the maximum, and the raised ureteral pressure was transmitted to the tubules via

the collecting ducts. The tubules would dilate into the intertubular spaces (Gottschalk, 1952, found an increase in intertubular pressure with ureteral ligation), collapse the blood vessels causing high resistance to blood flow, increase glomerular blood pressure, and hence, greater glomerular diameter (figure 44). Again, as with vein ligation, renal blood flow is diminished (Winton, 1951; Hinshaw et al., 1959; and Hinshaw et al., 1961), thereby decreasing glomerular filtration and, as a result, proximal tubule stenosis ensues (figure 45).

As shown in figure 30, thirty minute renal lymph duct ligation in the dog produces 45% increase in intertubular (interstitial) spaces. Lymph ducts are blind endothelial tubes, which terminate in the interstitium and partly function to relieve excess interstitial pressure consequent to filtration from capillaries. Here, lymph vessels draining the interstitial areas are obstructed and capillary filtration results in accumulation of fluid in the extracellular space and increased interstitial pressure. As hypothesized by Haddy et al. (1958), this pressure increase could cause peritubular capillary collapse and decreased renal blood flow. The resultant decreased glomerular filtration could cause, as observed in figures 30 and 46, proximal tubular

stenosis. In addition, as shown in Table 5, there is a significant decrease in outside diameters of all tubules.

As seen in figure 32, cat renal capsule removal produces tubular distension and stretching (compare to figure 24), Bowman's capsule and glomerular diameters are significantly decreased (Table 5), although intracapsular space (Bowman's capsule) is larger. Proximal (figure 47) and distal tubular inside diameters are significantly increased. These results are consistent with Brodie's (1914) findings in which he reported that renal capsule removal in the living cat produced an increase in tubular luminal and intracapsular (Bowman's capsule) spaces. One function of the renal capsule is probably to prevent overdistension of the turgid organ.

Intravenous adrenalin chloride (0.1 ml., 1:50,000) in rats produces some signs of kidney tubule stenosis (figures 33 and 45) and space depletion in about half of Bowman's capsules in a section. There is no significant change (Table 5) in glomerular (figure 44) or Bowman's capsular (figure 43) diameter, although intertubular space is decreased. The initial adrenalin slug probably acted primarily on the afferent glomerular arteriole (Ahlquist et al., 1954) to drop glomerular blood flow and filtration pressure causing proximal tubular stenosis. Later, the diluted,

recirculating adrenalin dilated the afferent and constricted the efferent glomerular arterioles (Richards and Plant, 1922 a & b). Subsequently, glomerular filtration pressure increased over the original and increased tubular pressure caused significant tubular distension. Since, at least one minute elapsed from injection to fixation by freezing, the slides probably represent the second phase of the adrenalin action in which the nephrons are recovering from the initial glomerular blood flow drop.

Intravenous histamine diphosphate (0.1 ml., 1:2,000) in rats produces proximal tubular stenosis (figures 34 and 45) and a marked drop in Bowman's capsular space. The result is very similar to renal artery ligation (figure 28), although with histamine interstitial space is less and red blood cells seem to be more abundant in capillaries. Also, there is a significant difference from controls in glomerular (figure 44) and Bowman's capsular (figure 43) diameters with histamine producing enlargment of both structures. Histamine causes a drop in systemic pressure (Bjering, 1937), and renal vasodilation (Page and McCubbin, 1953). Again, decreased glomerular blood flow and/or decreased glomerular blood pressure apparently caused proximal tubular narrowing. Renal blood flow, although apparently minimal, is adequate as indicated by normal glomerular size (figure 44) and the

red blood cell filled capillaries.

Diuretic rat kidneys (figure 35) show a significant increase in Bowman's capsular (figure 43), glomerular (figure 44), and internal (figure 45) and external tubular diameters (Table 5). The organ appears to be porous. Hypertonic glucose, used to produce diuresis, undoubtedly increased filtration and tubular pressures and caused tubular distension. The epithelium is thinned by stretching and there is little increase in brush border extension. Gottschalk and Mylle (1956) indicate this overall process when they reported increased intratubular pressure after hypertonic glucose injection.

After three weeks of hypertrophy, rat kidneys (figure 36) appear to be similar to controls but there are significant increases over controls in all nephron diameters (figures 43, 44, and 45, Table 5). This increase is probably due to cellular hypertrophy and/or cellular hyperplasia; no visual distinction can be made between the two processes.

Hypertensive rat kidneys (figure 37), produced by renal artery constriction (method "A"), show some stenosed tubules, a decrease in Bowman's intracapsular and intertubular space, and erythrocyte filled capillaries. Nephron diameters are significantly decreased over controls (Table 5, figures 43,

44, and 45) except distal tubules, and these are significantly greater than controls. Pearse (1961) believes the distal tubule is intimately concerned with renal hypertension as it shows a glucose-6-phosphodehydrogenase increase in distal tubules during early stages of the disease. The tubular hypertrophy is thought to indicate increased enzyme production.

Simultaneous collodion and gauze kidney wrap and contralateral nephrectomy (method "B") in rats (figure 38) produces striking, rapid results. Marked necrosis and foreign body reaction, from the collodion and gauze, prevails in the outer cortex. There are areas of both blood stasis and ischemia and the intertubular area is scanty. Nephron diameters (Table 5) are significantly less than hypertrophied controls, except the distal tubule diameter, and it is significantly greater than controls. Apparently, tubular hypertrophy into intertubular spaces, as theorized by Swann et al. (1959), pinches peritubular capillaries, raises renal blood flow resistance, and promotes renal ischemia.

Collodion and gauze wrap of hypertrophied rat kidneys (method "C") shows little difference from hypertrophied controls (compare figures 36 and 39), although there is a significant decrease in outer and inner proximal tubular and

outer distal tubular distance.

As shown in figure 40, hypertension in rats by collodion and gauze wrap followed seven days later by contralateral nephrectomy (method "D") produces a significant decrease (Table 5) in Bowman's capsular and glomerular diameters (figures 43 and 44). Intertubular space is decreased over hypertrophied kidneys and, as in method "B," there are areas of both blood stasis and ischemia. Tubular diameters are significantly less than hypertrophied controls. However, distal tubule inner diameter is significantly greater than hypertrophied controls.

Comparing the results of hypertension production, there appears to be a direct correlation between the restriction of renal distension and blood pressure increase over the control. An estimated twenty percent renal artery constriction by a silver clamp (method "A") caused a 28 mm. Hg systolic blood pressure increase. Collodion and gauze wrap of a hypertrophied kidney (method "C") causes a moderate distension restriction and a systolic blood pressure increase of 48 mm. Hg. Simultaneous collodion and gauze kidney wrap and contralateral nephrectomy (method "B") produces a severe distension restriction and a systolic blood pressure increase of 83 mm. Hg. However, if, as in method "D," one waits 7

days before removing the contralateral kidney, allowing the organ to remain and carry a portion of the excretion burden while the body and the wrapped kidney adjust to the collodion and gauze, the systolic blood pressure increase is only 53 mm. Hg. This indicates that perinephritis and restricted renal distension cause more pronounced renal hypertension than restricted renal distension alone.

Kidney sections from four-day adrenoprival rats (figure 41) show decreases in intertubular and proximal tubular luminal space. Bowman's capsular space is minimal. Bowman's capsular and glomerular diameters are significantly less than controls (figures 43 and 44). Proximal tubular and Henle's loop diameters are significantly greater than controls, although distal tubule diameters are significantly less than controls. Apparently, the conditions of hypotension and hypovolemia, as stated by Turner (1955) in adrenalectomized animals, produce these findings.

As seen in figure 42, rat kidneys, which were drained, frozen, cut, melted, dried, and stained, appear similar to conventional histologic slides (figure 17). Although very small intertubular and tubular luminal spaces were present, nuclear and cytological detail are unclear. In comparison to <u>in vivo</u> frozen-dried controls (figure 18), it appears

that considerable osmosis and diffusion could have taken place.

In conclusion, since the technique used here indicates the necessity for some revision in the classical, textbook concept of functional renal histology, it is suggested that these procedures might be applied profitably to the study of other organs.

SUMMARY

1. Kidneys of ten animal species were frozen, *in vivo*, with -175° C. isopentane, cut, lyophilized before thawing, stained, with alcoholic Wright's, and mounted; and contralateral kidneys were processed by conventional histologic methods. Comparison revealed marked differences with frozen-dried kidneys exhibiting wide tubular lumina, thin tubular epithelium, and large intertubular areas; and interdigitating cells appeared in larger tubule walls.

2. Ligation of rat renal artery, vein, or ureter or dog renal lymph ducts disturbs delicate intrarenal pressure relations to cause blood stasis and proximal tubular luminal stenosis. Renal lymph duct ligation also causes marked intertubular area increase.

3. Cat renal capsule removal produces tubular stretching indicative of overdistension. This is consistent with earlier findings. Apparently, the renal capsule prevents overdistension in the normal functioning state.

4. Concentrated doses of adrenalin or histamine in rats produce renal tubular luminal stenosis, but the secondary adrenalin effect, upon recirculation, produces tubular distension. Glucose, acting as a diuretic, significantly

distends and stretches tubules more than in "resting" kidneys.

5. Twenty-one day hypertrophied rat kidneys have significantly greater nephron diameters than controls. No distinction can be made between growth by simple hypertrophy or hyperplasia.

6. Seven day hypertension by renal artery constriction or perinephritis causes tubular luminal stenosis, a decrease in Bowman's intracapsular space and intertubular space, and blood stasis to a varying extent depending on the method used.

7. Four day adrenoprival rat kidneys showed decreased intraluminal and intertubular space. Apparently, hypotension and hypovolemia, associated with the syndrome, cause this condition.

8. Drained, frozen, cut, melted, and stained rat kidneys as prepared in this study, appear similar to the usual histologic preparations. However, the true compartmental relationships are distorted.

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