OBSERVATIONS ON THE FLUID CONTENT OF RAT KIDNEYS UNDER CONDITIONS OF HYPERTROPHY OR RESTRICTED FUNCTIONAL DISTENSION

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INTRODUCTION

Physiologists have known for many years that removal of one kidney leads to hypertrophy of the contralateral kidney (1). However, no one has determined whether or not the contralateral kidney will hypertrophy if it is enclosed in a snug capsule. This determination is of importance because encapsulation with collodion-soaked guaze, or other material, is a convenient, reliable way of inducing hypertension in experimental animals. Swann (2) has stated that hypertrophy does occur under these conditions, and considers that new tissue pre-empts the space formerly occupied by interstitial fluid and vascular blood in the kidney. He postulates that this reduction in "natural distension" is the fundamental hemodynamic change which causes hypertension.

This study was designed to test whether or not tissue mass in encapsulated kidneys increased following unilateral nephrectomy and to gain further information about the weight relations of renal tissue and fluids in both free and encapsulated kidneys.

HISTORICAL SURVEY

Hypertension was related to kidney function as long ago as 1845; Richard Bright stated that hypertrophy of the heart and hypertension were invariable findings in glomerulonephritis. This statement would naturally lead researchers to study the relation of hypertension and cardiac hypertrophy to renal function. Grawitz and Israel began this study in 1879. They removed portions of kidneys and observed that the heart did hypertrophy. Hypertension, they speculated, was the cause. In 1905, Passler and Heineke repeated the partial nephrectomy and measured a blood pressure rise. Thus, we have had experimental evidence that changes in the kidney could induce hypertension for 55 years.

The exact role of the kidney in hypertension, however, remains to be described. One difficulty has been to find an experimental method of altering renal physiology such that hypertension results. Many attempts along diverse lines (3) have been unsuccessful. In the same year (1905) that partial nephrectomy was shown to lead to an increase in blood pressure, Katzenstein did a partial occlusion experiment on the renal artery. Unfortunately, his period of observation seems to have been too short, and he reported equivocal results. In 1918, Bridgman and Hirose repeated this experiment and maintained the short observation period.

They reported negative results. Four years after Katzenstein's work, Alwens compressed both kidneys of cats with oncometers, but he, too, failed to produce a significant blood pressure rise. These failures persisted until 1932 when Saphir and Soskin wrapped one kidney in collodion-soaked gauze and removed the contralateral kidney (4). The authors clearly produced the pathologic symptoms of hypertension in their animals (necrotic arteriolitis, cerebral hyperemia, etc.), but did not measure blood pressure. Two years later Harry Goldblatt reaped the rewards of pioneering hypertension induction through altering renal circulation (5). His method was to reduce renal flow by partially clamping the renal artery. Despite the recognition he received, other investigators soon realized that his procedure had some limitations: hypertension was not always produced and quantitation of the degree of restriction placed on the artery was very difficult. Therefore, Irvine Page in 1939 tried several other methods (6) and found that un-oiled surgical silk, wrapped figure eight fashion, or a cellophane bag placed loosely around the kidney would more surely lead to hypertension.

Once renal dysfunction was securely established as a cause for hypertension, researchers began the attempt to identify the critical changes in the kidney. As is usually the case, an understanding of normal

function came to be recognized as the preliminary to an understanding of dysfunction. H. G. Swann and associates contributed to this understanding by their re-evaluation of the size and possible role of the interstitial space. First they showed that normal interstitial pressure was much higher (normally 25 mm.) than was previously believed (7). Then they gave good evidence that interstitial fluid occupied about 30 percent of the space in a normally distended kidney (8). Recently, Collings and Swann (9) did a study clearly demonstrating that albumin passes into the interstitial space rapidly. This indicated a relatively free, rather rapid circulation between blood and interstitial fluid. Swann et. al. (2) postulated that interstitial fluid plays an important role in renal physiology and possibly in hypertension of renal origin. It is, they believe, the fluid mediator and reservoir between the vascular system and the tubules.

Although no publication has made this point, Gottschalk and Mylle (10) seem to support this concept with their work on the countercurrent hypothesis of Hargitay and Kuhn (15). Briefly, the latter theory describes the effect fluid in the ascending and descending limbs of Henle's loop would have on each other if they were approximated. Assuming osmotic pressure to be the same in the two limbs, water would pass from the descending limb to the ascending limb because fluid in the

descending limb is under greater hydrostatic pressure. Therefore, the fluid which is in the descending limb at any given moment will become more concentrated. But as this concentrated fluid passes around the bend and up the ascending limb, fluid in the adjacent descending limb is not only under greater hydrostatic pressure, but also has a lower osmotic pressure; water leaves the descending limb and passes into the ascending limb. By this mechanism isosmotic ultra-filtrate from Bowman's capsule becomes more concentrated as it descends Henle's loop and more dilute as it ascends. Gottschalk and Mylle postulate active sodium reabsorption into interstitial fluid from the loop. They reason that the sodium produces a relatively high osmotic pressure in the medullary interstitial fluid of the kidney. The dilute fluid passing from the ascending limb into the distal convoluted tubule is hyposmotic with respect to the ultra-filtrate. However, as it passed down the duct, which is bathed in hyperosmotic interstitial fluid, it would become concentrated as water left the duct and entered the interstitial space. The vasa recta could act as "countercurrent diffusion exchangers" to help maintain the correct interstitial fluid osmolality.

Since the interstitial space seems to be so important to renal physiology, a more detailed examination of previous work in this area is warranted. Physiologists today visualize the kidney as an organ which changes

volume rapidly with changes in blood pressure. One group of experimenters (Wells and Replogle) conceive this expansion as being produced by venous resistance which is proximal to the arcuate veins and which, under increasing blood pressure, increasingly restricts venous out-flow. This causes increased renal intravascular pressure which is reflected in increased interstitial pressure (recall the free plasma movement between vasa recta and interstitial space). The increased interstitial pressure pushes on the capsule of the kidney and the kidney stretches. Others have studied total resistance variation of the kidney with blood pressure changes and have termed it autoregulation (12, 13). Whatever its cause, most researchers working on this problem agree that pressure-flow curves of renal blood are non-linear.

Swann apparently conceives of this kidney expansion as having the physiological function of increasing the quantity of interstitial fluid between tubules (2). He believes resorbate passes from the tubular wall into the interstitial fluid and thence into the renal blood stream. The interstitial fluid, it follows, is composed of plasma exudate and resorbate in transit for processing (14). Swann has done work which indicates that the plasma exudate is not greatly different from blood plasma.

Having arrived at the conclusions given in the last paragraph, the group from the University of Texas have

developed equations to yield total kidney blood and interstitial fluid volumes from the draining fluid (15). That is, if the renal artery is clamped and the renal vein cut, the mixture of fluid which drains from the kidney is composed of interstitial fluid and blood. The formula is: $V = \frac{H_A - H_K}{H_K}$ where V = interstitial fluid (diluting fluid) volume, H_A = systemic hematocrit, and H_K = renal vein hematocrit. This group has developed other formulae which they feel give approximations of protein concentrations in diluting fluid, the volume of resorbate in diluting fluid, etc., so that draining fluid in the kidney may be analyzed for concentrations, constituents, and volumes.

PROCEDURE AND MATERIALS

Male and female rats of the CFN (Carworth Farm Nelson) strain were used as experimental animals. Some of the females had been used as breeders for a short time, but neither males nor females had ever been used in any previous experiments. All weighed between 190 and 290 grams (Table 1) which corresponds to an age of about five months. The weights were read to the nearest five grams on a 500 gram capacity Toledo Balance.

The rats were subjected to one of two surgical procedures: simple unilateral nephrectomy, or unilateral nephrectomy and encapsulation of the contralateral kidney. Anesthesia was induced with intraperitoneal injections of 3 percent sodium pentobarbital. The males required 40 mg. per kg. of body weight, while the females reached a satisfactory surgical plane with only 30 mg. per kg. As soon as the rat was sufficiently anesthetized, an incision was made into the peritoneum just caudal and parallel to the seventh rib on the flank. The kidney was "popped" out to the surface and attachments of the capsule to the body wall were severed. If the capsule contained obvious fat depots, the fat and capsule were removed, but whenever possible, the capsule was left intact in order to help protect the parenchyma from the ether carrier in the collodion. The kidney at

this point was still attached to the rat by the renal vessels and the ureter, but was lying outside the body cavity. All manipulation of the rat or its kidney was then suspended for ten minutes so that the renal artery could re-distend the manipulated kidney to near its normal size. After this delay a hemostat was clamped around the pedicle and the entire stalk was tied by a single cotton thread proximal to the hemostat. The pedicle was then cut close to the hilus. An hematocrit was taken immediately from blood draining spontaneously from the cut renal vessels. The blood was drawn into a micro-hematocrit tube, sealed, and spun in an International Hemacrit Centrifuge for four minutes. The hemostat was removed and the stump checked for bleeding before the incision was closed. All muscles and the peritoneal layer of fascia were sutured in one layer, and the skin closed by an interrupted suture in a separate layer. The interrupted stitch was found to be necessary in order to prevent the rat from chewing out his stitches and opening the wound.

The removed kidney was allowed to drain about 30 seconds and a second hematocrit sample was drawn. After this hematocrit was taken, the kidney was allowed to drain ten minutes longer, and was then weighed in a five ml. beaker whose weight had been previously determined. All kidney weighing was done on a Voland & Sons chain-o-matic balance which measures to the nearest 0.1 mg.

Following weight determination, the kidney was placed in an oven which maintained a temperature between 100 and 120 degrees centigrade. Forty-eight hours later the dried kidney was removed from the oven and beaker and kidney were again weighed (it was found in a previous experiment that the tissues dried to constant weight in 48 hours).

After the incision was closed, the rat was kept in a warm room until it had regained consciousness, and was then transferred to an individual cage in an animal room which was maintained at a constant temperature of 73 degrees Fahrenheit. Here he had free access to water and food. The diet between procedures was always the same as prior to nephrectomy. For the next three days the rat was undisturbed. On the third post-operative day, he was again anesthetized, an incision was made in the other flank, and precisely the same procedure was followed as in the previous nephrectomy. The animal was sacrificed as soon as the kidney had been removed. From each rat, then, the following kidney data were taken: two hematocrits from a normal kidney and two from a kidney which had undergone three days of hypertrophy; the wet weight of a normal drained kidney and the wet weight of a drained hypertrophied kidney; and the dry weights of normal and hypertrophied kidneys.

The second group of rats was subject to a different surgical procedure. The first kidney was exposed and

freed from the body wall as before, but, instead of removing the kidney, it was wrapped in collodion-soaked gauze. The gauze (44 x 36 mesh bandage type) was cut into tiny rectangular strips and placed in a petri dish. Collodion Merck was poured into the dish, and the wrapping was begun immediately. The wet strips were laid on the kidney carefully so that every part of the kidney except the immediate area of the hilus was covered with two or more layers of gauze; the hilus was strictly avoided. About five minutes were allowed for the collodion to harden, and the kidney was then carefully re-inserted into the peritoneum in the same position it occupied before the procedure. Closing technique was identical with that already described for the nephrectomy. (The dried collodion gauze makes a very rigid non-expansible capsule. Swann has reported that this capsule shrinks 3 percent with hardening, and cannot be expanded by a dog's kidney (2).)

An important consideration in the wrapping technique was the condition of the kidney when the wrap was applied. If the kidney was under high vascular pressure, it would be greatly distended and the capsule would be large. If it was in an hypotensive condition, the applied capsule would be small (14). Since the wrapping technique is believed to induce hypertension because it restricts the dilation of the kidney, a hypotensive state seemed more desirable. Such a condition was thought to occur

immediately following manipulation of the kidney required to free it from the body wall: the organ was soft and flaccid. In addition, this condition would seem to be more reproducible than could be obtained if a distended state had been chosen. No blood pressure determination was made.

As soon as the flank incision had been closed over the wrapped kidney, the animal was turned over, and a nephrectomy was done on the other side. The nephrectomy was done following encapsulation instead of before it so that the kidney being wrapped would not have the full excretory load. Hematocrits and weights were taken on the removed kidney as was previously described. When the rat regained consciousness, he was returned to an individual cage in the animal room for three days.

After three days, rats were again given Nembutal, the wrapped kidney was exposed, and the ten minute delay period was observed. The pedicle was clamped and cut, and an hematocrit was taken immediately. The second hematocrit was not taken until the capsule had been removed because fluid drained very slowly from the encapsulated kidney. Weighing procedures were the same as with other kidneys. Therefore, each experimental animal subjected to the second procedure also yielded two hematocrits, two wet kidney weights, and two dried kidney weights.

RESULTS

Three basic groups of kidney data were obtained: wet weights, dry weights, and drained kidney fluid hematocrits. Within each group, data were obtained from normal rats, from rats unilaterally nephrectomized three days previously, and from rats with one kidney removed and the other one restricted by a snug collodion-gauze cast for three days. Since this study is essentially concerned with weight changes, the gains or losses in weight of the experimental kidneys over the controls are considered in the discussion more frequently than the actual weights. The expression "hypertrophied" or "compensating" will be used to designate the kidney which is free to expand (not wrapped) and which carried the full renal load of the rat for three days. The expressions "wrapped" or "encapsulated" will be used to specify the kidney which was placed in a collodion-soaked gauze capsule.

The tables listing data on kidney weights are numbers 2, 3, 4, and 5. The raw data are in the third and fifth columns of each table. Two corrections have been made on the raw data. One is a correction for the discrepency in weight between the left and right kidneys, and the other is designed to reduce all of the original kidney weights to an average weight. MacKay and MacKay (1) have reported that the right kidney is 3.0 percent heavier in male rats and 3.9 percent heavier in females.

The sex difference, they state, is not statistically significant. Therefore, in this study, each normal right kidney's weight was reduced by 3.4 percent and each normal left kidney's weights was increased by 3.4 percent. This correction has been applied in columns 4 of tables 2, 3, 4, and 5. In connection with this correction, it should be made perfectly clear that MacKay et. al. made their observations on drained wet kidneys and that the weight corrections in this paper were applied to both drained wet kidneys and dried kidneys. Therefore, it was assumed here that the left and the right kidneys drain proportionately. In columns 6 the gains in weight are listed. These gains represent the changes in weight induced by the experimental procedures. The second correction of kidney weights mentioned above makes the weight gain independent of the original size of the kidney. For example, in table 2, the corrected wet kidney weight of animal #1 was 845.0 mg., the average corrected wet weight of all the normal kidneys was 904.5 mg. (see Table 7), and the compensating drained kidney gained 81.2 mg. Therefore, 904.5 x 81.2/ 845.0 = 86.9. All weight gain values, corrected in this manner, are given in the last column of each table.

In tables 3, 4, and 5, one or two corrected gains are marked with an asterisk. These data are more than three standard errors from the mean. It is felt that such a large variation indicates some process which did

not occur with the other animals. Consequently, these data were not included in the calculations.

The wet hypertrophied (compensating) kidneys showed a mean gain of 159.4 mg. ± 81.6 mg. over the weight of the normal wet kidneys (Table 2). Wet wrapped kidneys (Table 3) had a mean gain of 112.8 mg. ± 69.5 mg. over the normal wet kidneys. In order to test whether or not the above two samples were part of the same population, a "t" test was done (See Appendix 1). It failed to show that the two samples were significantly different at the 95 percent confidence level.

A correlation coefficient was determined (See Appendix 1) comparing the individual wet weight of the wrapped kidney to its wet weight gain. This amounted to a test to see whether or not the larger wrapped kidneys gained more wet weight than the smaller ones. The coefficient was .202, which is not significant.

Tables 4 and 5 are based on dried kidney weights. Table 4 compares the weights of dried normal kidneys to the weights of dried hypertrophied kidneys. They gained an average of 25.4 mg. ± 11.0 mg. Table 5 compares dried normal kidney weights to weights of dried kidneys which had been wrapped. The mean gain of these kidneys was -0.3 mg. ± 9.3 mg. This mean gain of the sample was not statistically different from 0 (See Appendix 1). The "t" test value (8.10) indicated that dry weight gains in wrapped kidneys were significantly different from dry

weight gains in compensating (not wrapped) kidneys.

Two more correlation coefficients were determined on the kidney weight data. One was the correlation between the wet and dried weight gains of the hypertrophied kidneys. The coefficient (0.49) was significant at the 95 percent level. The other correlation coefficient was between the wet and dried weight gains of the wrapped kidneys. This coefficient was 0.71. The meaning of these will be discussed below.

Table 6 contains all of the hematocrit data. The statistical data based on hematocrits are given below and Appendix 1 shows the formulae used in obtaining them. The mean of all first hematocrits from normal kidneys was 30.9 $\pm$ 8.8. The first hematocrit on the hypertrophied kidneys' fluid gave a mean of 27.8 $\pm$ 7.7. Wrapped kidneys had mean hematocrits of 39.9 $\pm$ 7.9. "t" tests comparing the first hematocrits of the above samples (normals, hypertrophied, and wrapped) indicated that normal and wrapped kidney hematocrits were from a different population than first hematocrits from the hypertrophied kidneys at only a 90 percent confidence level.

Similar statistics were computed for the second hematocrits. The mean of second hematocrits was 15.7 $\pm$ 7.1 for normal kidneys, 11.2 $\pm$ 5.5 for compensating kidneys, and 18.6 $\pm$ 5.6 for wrapped kidneys. In comparing these, only the hematocrits of wrapped kidneys vs. normal

kidneys was statistically different.

One important consideration which has not been mentioned is the gross physical condition of the whole rat. The assumption was made that the wrapping procedure induced hypertension because it did in other experimental animals (2,3) and because the manifest symptoms were similar. However, no blood pressure readings were taken. All rats subjected to unilateral nephrectomy and contralateral collodion-soaked gauze wrapping technique appeared to be quite ill by the second post-operative day. Their fur was ruffled, they ate little or nothing, and they were much less active than the normal rat of this size and strain. Occasionally epistaxis and diarrhea had appeared by the second day. By the third day, those rats which survived appeared rather comatose, most had epistaxis and diarrhea, all had ruffled fur, and probably all had lost weight. The weight loss of a random sample of the surviving animals was 15-35 gm.

21 of 41 rats whose kidneys were wrapped died after recovery from anesthesia but before the three-day delay period was finished. Several of these animals were posted. The results were unusual, largely because they were not remarkable. The gross pathology of these animals was not different from that in animals surviving three days. Both groups had begun to wall-off the collodion-soaked gauze with mesentery, panniculus

adiposus, and even intestine. The space between the capsule and the wall was filled with a watery fluid. All posted rats had some degree of ecchymosis in the abdominal viscera - frequently the visceral fluid was obviously bloody.

The kidney itself was not obvicusly damaged. Some fibro-elastic connective tissue had lightly connected the capsule to the kidney, and some small superficial hemorrhagic spots were visible on the surface of the kidney, but otherwise, the kidney appeared normal. One kidney was sectioned, and slides prepared for a microscopic examination. The slides showed only moderate hyperemia in an otherwise normal appearing kidney section. There was no evidence of a marked inflammatory process.

DISCUSSION

The data on hypertrophied kidneys support the results of Mousstgaard on dogs (16) and MacKay and MacKay on rats (1); both the wet drained kidneys and the dried kidneys gained weight significantly. In addition, the data from this experiment show that the dry weight change was roughly proportional to the change in wet weight. Therefore, it appears that the unrestricted kidneys hypertrophied to compensate for the loss of the contralateral kidneys.

	Normal	Hyper.	Wrapped
Mean wet weight (mg.)	904.5	1063.5	1017.3
Mean dry weight (mg.)	194.4	219.8	194.1
Percent fluid	78.5	79.3	80.9
Mean wet weight gain over normal		159.4	112.8
Mean dry weight gain over normal		25.4	-0.3
Mean first Hct.	30.9	27.8	37.9
Mean second Hct.	15.7	11.2	18.6

Table 7

Table 7 above is a convenient reference for the discussion to follow.

The wet wet weight changes of wrapped kidneys are compatible with Swann's data on dogs (2); a significant gain (112.8 mg.) over the control weight was observed. The dry weight of wrapped kidneys, however, is not what

would be predicted by Swann's conclusion (2): true tissue mass increase after wrapping would have appeared as a dry weight gain just as it did in the hypertrophied kidneys. However, no gain in dry weight of the wrapped kidneys appeared in this experiment.

Swann's theory of a reduction in "functional distension" as the fundamental cause of hypertension (2) would seem to be unfounded. The gain in weight of wrapped kidneys on which he based his theory apparently is due to fluid increase and not to a dry tissue mass increase. The theory could be essentially correct, of course, and not contradicted by the present work. Since it was believed that the kidneys in this experiment were wrapped while in a non-functional, undistended state, and since they cannot distend after the collodion-gauze cast has hardened, these kidneys quite possibly contain reduced quantities of renal fluid. The data here, then, would suggest that any reduction in natural distension occurred during manipulation and wrapping of the kidney, and not as the result of tissue hypertrophy.

It is intriguing to consider Gottschalk's and Mylle's countercurrent hypothesis (10) as it relates to the problem of a necessary minimum kidney distension. These authors seem to infer that an osmotic gradient exists in the interstitial fluid. They visualize interstitial fluid as a medium which

relays electrolyte concentration information to adjacent tissues and transports material from tubules to blood vessels. It is not unreasonable to speculate that limbs of Henle's loops may be too close to each other in an undistended kidney or that osmotic gradients in interstitial fluid become abnormal when the tissues are crowded together. Perhaps the reason "natural distension" is vital to normal kidney function will be found from research on these possibilities.

The data from this experiment showed that wrapped kidneys did not gain in dry weight. However, the sizable wet weight gained by encapsulated kidneys should be explained. The present experiment is inadequate for this, but some speculation on the subject can be based on an analysis of the data. If the renal cells became hydrated as a result of the wrapping procedure, the wet weight gain would be explained. However, with hydration, we would expect the larger kidneys to show a larger wet weight gain. The correlation between the wet weight of the individual wrapped kidney and its wet weight gain was .202. This correlation coefficient gives a "t" value of 1.24 which is not significant. Therefore, we are not justified in assuming that cell hydration explains the wet weight increase.

One other possibility which would not fully explain all of the data but which would explain the high correlation coefficient (0.71) between wrapped wet weight

gain and wrapped dry weight gain of the animals should be mentioned. Saphir and Soskin (4) cut windows in their gauze kidney wrappings and the kidney bulged out this opening. Possibly variations in technique allowed more or less bulging around the hilus of the kidney in this experiment, the bulging was true hypertrophy, and this produced the correlation between wet and dry weight gains of the wrapped kidneys. (Obviously, the mean weight gain of the wrapped kidneys is not explained by this hypothesis.) If variations in weight were produced in this random manner, the actual weight of the wrapped kidney should correlate closely with its weight gain over the contralateral kidney. As given in the preceding paragraph, this correlation coefficient was only .202. Therefore, it is unlikely that wrapping technique variation explains the high correlation between wet and dry weight gains of the wrapped kidneys.

This high correlation coefficient between wet and dry weight gains of wrapped kidneys is very interesting. The corresponding correlation between wet and dry weight gains of hypertrophied kidneys is only .49 and yet the obvious explanation is that the renal tissue hypertrophies and therefore retains more total cellular water. With wrapped kidneys, a very small weight change (mean -0.3 mg.) seems to produce a great (112.8 mg.) fluid retention. Whether the explanation for this will derive

from the work of Wells and Replogle (11) on venous resistance, from the work of Swann (17) on arcuate vein cushions, or from an entirely different source cannot be indicated from the data of this experiment.

Let us leave the data on kidney weights and examine the hematocrit data. Note the means of the first hematocrits of each group from Table 7; hypertrophied 27.8, normal 30.9, and wrapped 37.6. Since body hematocrits were not taken, no comparison to the usual whole body hematocrit is warranted, nor is there any reason to expect it to be unusual (see Swann, 2). However, one could speculate that the wrapped kidney's capsule was under less tension than the normal kidney, and the normal kidney capsule was under less tension than the hypertrophied kidney's capsule. Such an assumption fits the data. Since this paper has pointed out that the wrapping technique employed in this experiment has produced hypertension for other workers (2,3), and is assumed to do the same here, the question might arise why an elevated blood pressure does not produce an increased tension on the capsule of the wrapped kidneys. Recall that the wrap (called a cast by Saphir and Soskin) is very firm and inflexible. Since the wrap was believed to have been applied when the kidney was in an undilated condition and since collodion shrinks the cast about 3 percent (2), pressure exerted by interstitial fluid on the capsule cannot stretch the capsule but is transmitted to the

wrap. Therefore, wrapped kidneys are under high pressure, but tension in the capsule is low.

Assuming, then, that capsular tension was least in the wrapped kidneys, intermediate in the normal kidneys, and greatest in the hypertrophied kidneys, the hematocrit data becomes comprehensible. Having no ability to dilate, the wrapped kidneys had relatively little interstitial fluid and relatively little capsular tension to force interstitial fluid into the capillaries and out the renal vein once arterial pressure was removed by clamping and cutting the renal artery. Both of these factors caused the first hematocrit of the wrapped kidneys to be high.

The hypertrophied kidneys were assumed to be under greater tension than the normal kidneys. No evidence has been cited for such as assumption, although we may be tempted to feel that hypertrophy may have been induced by slightly elevated blood pressure. The data in this experiment do not show a significant difference at the 95 percent confidence level between normal and hypertrophied kidney first hematocrits, ($t = 1.34$). However, the tendency would be to support an hypothesis that hypertrophied kidney capsules were under greater tension than normal kidney capsules and that when the renal artery and vein were severed and opened to the atmosphere, more interstitial fluid was forced into the capillaries and out the renal pedicle. This

increased quantity of interstitial fluid, with its near lack of RBCs, would produce a lower hematocrit for hypertrophied kidney fluid than for normal kidney fluid.

The second hematocrits also support the hypothesis of reduced capsular tension in the wrapped kidneys and increased capsular tension in the hypertrophied kidneys. When pressure is removed from the capsule by opening the renal artery and vein to the atmosphere, a very tense capsule would force more fluid from the kidney, and, in a manner of speaking, rinse the blood out of the vascular tree. Therefore, following drainage, less red blood cells would be left to contribute to the hematocrit. Similarly, a capsule (like those of the wrapped kidneys) under low tension would wash out fewer red blood cells and the second hematocrit would still be elevated over the second hematocrit of the normal kidney. Actually, wrapped kidneys were observed to drain less than both normal and hypertrophied kidneys.

SUMMARY

Twenty rats were nephrectomized; the contralateral kidneys were undisturbed for three days, and then they were removed. Twenty other rats were nephrectomized; the contralateral kidneys were wrapped with collodion-soaked gauze and returned to their original positions for three days, and then they were removed. Hematocrits were taken from the first fluid to drain from the pedicle of each kidney and from the last fluid to drain from the pedicles. The drained kidneys were weighed, dried 48 hours, and then re-weighed.

Both the drained and the dried weights of the single, undisturbed kidneys increased significantly over the weights of their excised mates (159.4 mg. and 25.4 mg. respectively). Apparently these kidneys hypertrophied. The drained weights of the wrapped kidneys also increased significantly over the weights of the normal contralateral kidneys (112.8 mg.). The dried weights of wrapped kidneys, however, did not increase over the dried weights of normal kidneys (-0.3 mg). The increased weight of drained wrapped kidneys was concluded to be fluid which was retained and not hypertrophy as has been indicated in other work. Some statistical, indirect evidence was presented indicating that the fluid was not intracellular.

A very high correlation between wet weight and

dried weight of the wrapped kidneys was noted. It is believed that this proves that a very small change in renal tissue mass causes, or accompanies, large changes in the quantity of fluid retained in wrapped, drained kidneys.

Data from both the first (normal 27.8, wrapped 37.9, and hypertrophied 30.9) and the second (normal 11.2, wrapped 18.6, and hypertrophied 15.7) hematocrits of renal draining fluid could be explained by postulating that the capsules of hypertrophied kidneys were under more tension than normal kidneys and that the capsules of wrapped kidneys were less tense than normal because arterial pressure was transmitted to the collodion-gauze cast.

Table 1

Weight and Sex Data on Rats

No.	Sex	Weight (gm.)	No.	Sex	Weight (gm.)
1.	F	215	1.	F	265
2.	F	270	2.	F	265
3.	F	235	3.	F	270
4.	F	285	4.	F	190
5.	F	280	5.	F	260
6.	F	260	6.	F	220
7.	F	250	7.	M	255
8.	M	235	8.	M	230
9.	F	280	9.	M	295
10.	F	250	10.	M	245
11.	M	215	11.	M	240
12.	F	325	12.	M	205
13.	F	200	13.	F	235
14.	F	235	14.	F	245
15.	M	215	15.	F	265
16.	M	205	16.	F	235
17.	M	285	17.	F	235
18.	M	265	18.	F	265
19.	M	270	19.	F	260
20.	M	280	20.	M	255

The data on the left side of the table are from rats which gave normal and hypertrophied kidney data. The right hand columns are from rats which gave normal and wrapped kidney data.

Table 2

Comparison of the Wet Weights of Compensating Kidneys to Wet Weights of Normal Kidneys (mg.)

No.	Normal left-right	weight	Corr. normal	Compensating	Gain	Corr. Gain
1.	R	874.7	845.0	955.9	81.2	86.9
2.	R	1036.5	1001.3	1277.4	276.1	249.4
3.	R	847.0	818.2	1016.2	198.0	218.9
4.	L	942.4	974.4	1139.0	164.6	152.8
5.	R	939.5	903.7	1028.0	124.3	124.4
6.	L	762.3	788.2	886.4	98.2	112.7
7.	R	909.8	878.9	1270.9	392.0	404.0 *
8.	R	951.8	919.4	1053.3	133.9	131.7
9.	R	1084.7	1047.8	1564.8	517.0	445.9 *
10.	L	951.2	983.5	1120.7	137.2	124.1
11.	L	803.1	830.4	917.0	86.6	95.4
12.	L	1072.0	1108.4	1144.8	36.4	29.7
13.	L	672.5	695.4	941.1	245.7	319.6
14.	R	783.3	756.7	971.8	215.1	257.1
15.	R	843.6	814.9	937.4	122.5	136.0
16.	R	735.3	710.3	863.0	152.7	194.4
17.	R	938.4	906.5	1193.2	286.7	286.1
18.	L	931.4	963.1	1181.6	218.5	205.2
19.	R	1009.2	974.9	1053.6	78.7	73.0
20.	L	1147.4	1186.4	1282.5	96.1	73.3
					mean =	159.4
					standard error =	81.6

* not included in calculations

Table 3

Comparison of the Wet Weights of Wrapped Kidneys to Wet Weights of Normal Kidneys (mg.)

No.	Normal left-right	Normal weight	Corr. normal	Wrapped	Gain	Corr. Gain
1.	L	874.2	903.9	1043.8	139.9	140.0
2.	L	858.3	887.5	1050.3	162.8	165.9
3.	L	929.8	961.4	1061.7	100.3	94.4
4.	L	777.9	804.3	829.0	24.7	27.8
5.	R	953.1	920.7	1049.3	128.6	126.3
6.	R	651.7	629.5	775.2	145.7	209.3
7.	L	1133.5	1172.1	1024.0	-148.1	-114.1*
8.	R	986.3	952.8	1112.7	159.9	151.8
9.	L	1038.0	1073.3	1101.1	27.8	23.4
10.	L	873.9	903.6	1082.1	178.5	178.6
11.	R	967.7	934.8	927.1	-7.7	-7.5
12.	L	762.3	788.2	911.7	123.5	141.7
13.	L	756.3	782.0	868.7	86.7	100.3
14.	R	931.2	899.5	1026.3	126.8	127.5
15.	L	796.6	823.7	904.5	80.8	88.7
16.	R	716.9	692.2	849.5	157.3	205.5
17.	R	841.2	812.6	874.4	61.8	68.8
18.	L	951.6	984.0	1000.0	16.0	14.7
19.	R	879.6	849.7	1063.8	214.1	227.9
20.	R	1096.5	1059.2	1126.5	67.3	57.5
					mean =	112.8
					standard error =	69.5

* not included in calculations

Table 4

Comparison of the Weights of Dried Compensating Kidneys to Weights of Dried Normal Kidneys (mg.)

No.	Normal left-right	weight	normal	Compen-sating	Gain	Corr. Gain
1.	R	205.1	199.1	210.4	11.3	11.0
2.	R	277.3	268.9	315.0	46.1	31.1
3.	R	206.6	199.6	214.9	15.3	13.8
4.	L	213.4	222.7	258.5	35.8	33.6
5.	R	213.9	206.6	221.8	15.2	14.2
6.	L	180.3	186.4	194.6	8.2	8.5
7.	R	206.8	199.8	239.9	40.1	38.8
8.	R	207.5	200.4	222.1	21.7	20.9
9.	R	222.3	214.7	235.5	20.8	19.0
10.	L	158.8	164.2	185.6	21.4	25.2
11.	L	174.3	180.2	194.1	13.9	14.9
12.	L	188.6	195.6	218.2	22.6	22.3
13.	L	143.6	148.5	180.3	31.8	41.4
14.	R	174.7	168.8	204.6	35.8	35.0
15.	R	173.1	167.2	191.2	24.0	27.7
16.	R	160.6	155.1	190.6	35.5	44.2
17.	R	204.2	197.3	216.1	18.8	18.4
18.	L	193.8	199.7	243.1	43.4	42.0
19.	R	213.2	206.0	236.1	30.1	28.2
20.	L	239.9	248.2	270.8	22.6	17.6
					mean =	25.4
					standard error =	11.0

Table 5

Comparison of the Weights of Dried Wrapped Kidneys to Weight of Dried Normal Kidneys (mg.)

No.	Normal left-right	weight	Corr. normal	Wrapped	Gain	Corr. Gain
1.	L	196.3	203.0	204.3	1.3	1.2
2.	L	195.9	202.6	199.1	-3.5	-3.3
3.	L	200.7	207.5	206.6	-.9	-.8
4.	L	163.3	168.8	165.7	-3.1	-3.5
5.	R	197.2	190.5	196.8	6.8	6.9
6.	R	148.2	143.2	161.2	18.0	24.3
7.	L	243.7	252.0	207.7	-44.3	-34.0*
8.	R	182.4	176.2	171.0	-5.2	-5.7
9.	L	235.2	243.2	232.9	-10.3	-8.2
10.	L	193.6	200.2	205.2	5.0	4.8
11.	R	201.4	194.6	182.0	-12.6	-12.5
12.	L	163.3	168.8	166.4	-2.4	-2.7
13.	L	158.7	164.1	161.9	-2.3	-2.7
14.	R	195.2	188.6	198.8	10.2	10.4
15.	L	172.2	178.1	180.1	2.0	2.2
16.	R	173.7	167.8	167.9	.1	.1
17.	R	184.1	177.8	168.1	-9.7	-10.5
18.	L	198.9	205.7	187.6	-16.9	-16.9
19.	R	190.1	183.6	193.5	9.9	10.4
20.	R	226.6	218.9	218.8	-.1	-.1
					mean =	-.3
					standard error =	7.6

* not included in calculations

Table 6

Renal Fluid Hematocrits
(percent)

	Normal		Compensating			Normal		Wrapped	
No.	1st. Hct.	2nd. Hct.	1st. Hct.	2nd. Hct.	No.	1st. Hct.	2nd. Hct.	1st. Hct.	2nd. Hct.
1.			18	10	1.	38	16	37	17
2.			20	9	2.	36	13	41	16
3.	44	34	20	11	3.	41	11		8
4.	41	27	33	19	4.	33	13	33	11
5.	20	11	29	16	5.	34	14	27	6
6.	40	30	40	23	6.	33		27	16
7.	20	12	34	12	7.	38	26	27	19
8.	35	15	26	8	8.	18	8	31	15
9.	28	16	38	20	9.	39	22	45	27
10.	35	18	14	4	10.	25	11	30	21
11.	19	7	33	6	11.	29	15	50	25
12.	45	24	34	13	12.	26	10	52	20
13.	21	11	25	7	13.	40	17	39	23
14.	41	22	21	10	14.	24	16	33	20
15.	29	16	23	8	15.	35	11	46	23
16.	31	13	22	8	16.	49	35	42	21
17.	23	10	40	21	17.	26	13	38	18
18.	19	9	32	5	18.	19	10	40	27
19.	18	9	23	10	19.	23	10	45	20
20.	30	11	20	5	20.	—	—	—	—
		MEAN =	27.8	11.2		30.9	15.7	37.9	18.6
		Standard Error =	7.7	5.5		8.8	7.1	7.9	5.6

Appendix 1

Formulae Used in Analyzing Data

Standard Error of the Mean:

$$s_x^2 = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}$$

"t" values to test whether or not two samples are part of the same population:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{s_x^2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

"t" values to test whether or not one sample is different from 0:

$$t = \frac{\bar{X} - 0}{\sqrt{s_x^2 \left(\frac{1}{n} + 0\right)}}$$

Correlation between two samples:

$$r_{xy} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right)\left(\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right)}}.$$

"t" values to test the significance of a correlation:

$$t = \frac{r_{xy}\sqrt{n-2}}{\sqrt{1 - r_{xy}^2}}$$

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