THE FUNCTIONAL CAPACITY OF HYPERTROPHIED NEPHRONS

Effect of Partial Nephrectomy on the Clearance of Inulin and PAH in the Rat*

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Chronic renal disease in man, whether it be the result of chronic glomerular nephritis, chronic pyelonephritis, or vascular disease, is associated with a loss of total nephron mass (1). Patients with chronic renal disease maintain renal function only to the extent that the remaining nephrons are able to compensate for those which have disappeared. The investigation of this process of functional compensation which accompanies the reduction in renal mass is critical to an understanding of chronic renal insufficiency. There are many reports of measurements of renal function in patients with chronic renal disease; these have been made by conventional clearance methods (2–4). In general the results of these studies indicate the residual glomeruli show a greater compensatory activity in response to the decrease in nephron population than do the residual tubules which are left. In none of these studies were the functional measurements coupled with determination of the amount of renal tissue remaining at autopsy, and the only information bearing on this point is contained in a report by Hayman, Martin, and Miller (5), who compared creatinine clearance in patients with chronic Bright's disease with the number of glomeruli found in their kidneys at autopsy.

The extent to which remaining nephrons can compensate for a reduction in their number has also been studied in animals subjected to partial nephrectomy. In partially nephrectomized dogs, which develop raised blood urea levels and polyuria if sufficient renal tissue be removed (6), glomerular filtration rate, measured either by creatinine clearance or inulin clearance, is not reduced to as great an extent as would be expected considering the amount of renal tissue removed (7–10). Findings of a similar nature were reported in the partially nephrectomized rat where the creatinine clearance is greater than would be expected in view of the amount of loss of renal tissue (11); it should be noted, however, that creatinine clearance is an uncertain measure of glomerular filtration in the rat. None of these studies in animals so far has

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included a comparison of the number of glomeruli remaining in the kidneys with the functional data in the corresponding animals.

With this in mind, it was decided to study the changes in renal function which follow partial nephrectomy in rats using each animal as its own control, and correlating the changes in some of the rats with the number of glomeruli remaining in the kidneys. We report here sequential measurements of inulin and paraminohippurate (PAH) clearance in rats made before and for a long period after partial nephrectomy together with counts of the number of glomeruli in the kidneys of some of the rats.

Materials and Methods

General Design of the Study.—Three groups of male Wistar rats obtained from Carworth Farms, New City, New York, were used in the study. The rats of the first group were partially nephrectomized by a technique described in detail elsewhere (12). A sham operation was carried out on those of the second group; the animals in the third group were not operated upon and served as controls. Some rats of each group were used for inulin clearance studies only, while the remaining rats in each group had PAH measurements made as well.

The work was carried out in two stages. In the first stage an investigation of inulin clearance of the partially nephrectomized rats, the sham-operated controls, and the normal controls was made. Inulin clearance measurements were made on almost all rats before the operation when they weighed between 150 and 200 g. Further inulin clearance determinations were made on the nonoperated controls and sham-operated controls following the operation, and inulin clearances were determined in most of the rats in these groups at intervals of 4 to 5, 7 to 11, 12 to 14, 15 to 17, 27 to 30, and 43 to 50 wk after the operation. We had 16 sham-operated control rats and 10 nonoperated control rats available for this part of the experiment.

Since we were unsure of the ability of the partially nephrectomized rats to withstand repeated peritoneal infusions of the inulin solution which is used for clearance determinations, all the experimental animals were not subjected to a clearance measurement at each interval. Some of the experimental animals were used for the 4 to 5 wk postoperative measurements, and no further measurement was made until 12 wk after operation, while no clearance studies were made on others until 15 or 16 wk after operation. This part of the experiment started with 21 partially nephrectomized rats, some of which died as the experiment proceeded. Seven of the animals survived at least 43 wk after operation, at which time inulin clearance measurements were made.

In the second stage of the work the experience gained with peritoneal infusion of partially nephrectomized rats allowed us to carry through inulin and PAH measurements in each of 9 partially nephrectomized rats and 9 sham-operated controls at intervals of 1, 4, 8, 12, and 24 wk after operation. At the end of the series of clearance measurements some animals of each group were killed and the kidneys examined histologically by conventional methods.

Operations.—The partial nephrectomy was carried out in two operations performed a week apart. At the first the upper and lower poles of the left kidney were removed, leaving about one third of the kidney behind. At the second operation the whole right kidney was removed. In sham operations the kidney capsule was stripped, but no renal substance was removed.

Clearance Procedure.—A male rat of the Carworth Farm (CFN) Wistar strain was anesthetized with ether and placed supine so that a V-shaped rack (Fig. 1) could be taped to the hind legs. A 17 gauge, 2 in. hypodermic needle was passed percutaneously into the peritoneal cavity, being directed from the lower left abdominal quadrant medially and toward the upper right quadrant. Approximately 6 in. of polyethylene tubing (Intramedic PE 50, ID-0.023 in.) was passed through the needle into the abdominal cavity and then the needle carefully withdrawn over the catheter, leaving it in place. Collodion was daubed around the tubing to seal the opening, and a piece of tape was placed to further secure the catheter to the rat's body. To assure that the tubing would not be subsequently compressed, it was placed between the animal's body and the V rack, passing below the foot. Anesthesia was now discontinued and the rat, taped to the rack, was placed inside the restraining cage in a prone position so that the penis projected through the bottom slot of the cage.

The cage was placed on a collecting stand so that the rat's penis which projected through the slot was directly over a beaker placed below. At this time a nick was made in the rat's tail with a sharp scalpel and a blood sample (0.2 ml) collected in a Natelson microcollecting tube. The rat was not anesthetized for this procedure. A priming dose of the substance of which the clearance was being determined was injected through the polyethylene tubing into the peritoneum. When inulin clearances alone were being determined, this dose consisted of a 6% solution of inulin in 0.6% NaCl. When PAH clearances were being determined as well, 250 mg of PAH were incorporated in each 100 cc of the inulin infusion fluid. The amount of fluid given depended upon the type of rat and its weight. In nonoperated and sham-operated control rats the volume of primer in ml was calculated by the formula (body weight in grams)/120, and in partially nephrectomized rats the formula used was (body weight in grams)/240.

After priming, a constant sustaining infusion was started using the Harvard infusion pump. The ideal rate of infusion for all rats in ml/min was obtained by the following formula: (body weight in grams)/10,000. The rate closest to this value which it was possible to deliver with the pump was the one used. In nonoperated or sham-operated controls when inulin clearances alone were being determined, a 3% solution of inulin in a 0.6% NaCl was used, and 250 mg of PAH per 100 ml were added to this when PAH clearances were being determined as well. In partially nephrectomized rats a 2% inulin solution in a 0.6% NaCl was used for inulin clearances alone, and 250 mg PAH was added to this solution per 100 ml when PAH clearances were under investigation.

The sustaining infusion was allowed to run for at least 90 min, which served as an equilibration period. During this time urine flow increased greatly, and the rats voided spontaneously three or four times. At the end of the 90 min equilibration period the rats were carefully watched, and the time of the next spontaneous voiding was noted. The urine was discarded, a fresh clean beaker was placed below the rat and the collection period started. A tail blood sample was obtained within a minute or two after the voiding. In some rats spontaneous voiding did not take place within 10 or so minutes of the end of the 90 min equilibration period, and in these animals voiding was readily induced by cutting the tail for the blood sample.

The test period continued for about 90 min, and the urine was collected during the whole period. At the end of the test period the rats were again closely observed for a spontaneous urine voiding, the time of which was noted. At this point finger pressure above the symphysis pubis and over the bladder was exerted to ensure complete emptying of the bladder. In none of the animals was additional urine ever expressed by this means. This constituted the end of the test period, and no further urine was collected. A blood sample was again obtained from the tail within a minute or so of this voiding. In none of the animals did this procedure induce voiding of additional urine, a result which confirms our belief that emptying of the bladder was complete. The total volume of urine collected during the test period was measured. Serum and urine were analyzed for inulin and PAH respectively, and calculations of inulin and PAH clearance were made using standard formulae well described in standard texts. No correction for PAH extraction was introduced into the value for the PAH clearance.

Chemical Methods.—Analyses for inulin in the serum and in the urine were made by the resorcinol method of Bacon and Bell (13) as modified by Higashi and Peters (14). PAH was measured on 0.1 ml aliquots of serum and appropriately diluted urine by the method described by Smith, Finkelstein, Aliminosa, Crawford, and Graber (15) modified (16) by reducing twentyfold the volumes of all reagents used.

Extraction Ratio of PAH.—This was measured in 2 partially nephrectomized rats and 5 sham-operated rats of the second series of animals when all clearances had been completed. PAH extraction ratios were also measured in a group of nonoperated controls which had not had clearance studies, as well as on four partially nephrectomized rats which were specially prepared for the purpose. A solution of PAH in saline was injected into the femoral vein in sufficient amount to raise the blood level to approximate the level which is ordinarily present during PAH clearance determinations. 10 min after injection the animals were anesthetized with ether, the abdominal wall opened, and simultaneous renal vein and aortic blood samples taken. In order to avoid errors due to entry of blood into the left renal vein from the adrenal and testicular veins of the same side, both of these vessels were ligated. Also as soon as the hypodermic needle was introduced into the renal vein, this vein also was clamped off between the point where the needle entered the vein and the place where the vein joined the vena cava. This prevented caval blood from flowing back into the renal vein during sampling. The sera from the two samples were analyzed for PAH. A comparison of the level of PAH in the aorta and in the renal vein gave the extraction ratio of PAH for the kidney.

Measurement of Entry of PAH into Red Cells.—Blood was drawn by aortic puncture from previously heparinized rats. A 10 ml aliquot of blood was taken, and 0.1 ml of a 0.1% solution of PAH in 0.9% saline was added to it and well mixed. From this 1 ml aliquots were withdrawn at 5, 15, 30 min, and 1 and 17 hr intervals and centrifuged. The hematocrit was measured in each aliquot, and PAH determinations were run on the supernatant plasma. The stock of blood was kept well mixed during the experiment.

Glomerular Counts.—Glomerular counts were made on the kidneys of rats in each of the three groups. Only a small number of animals from each group was used for this because the method was not satisfactorily worked out for use until late in the study. The counts were made by the method of Kunkel (17).

RESULTS

The results of the earlier part of the study in which inulin clearances were measured at successive intervals in nonoperated controls and at selected postoperative intervals in the partially nephrectomized rats and sham-operated controls are presented in Table I. In Table II there are set out the results of the second part of the study. These consist of the simultaneous clearances of inulin and of PAH at low plasma PAH concentrations (less than 1.0 mg per 100 ml) in a group of sham-operated controls and a group of partially nephrectomized rats at selected intervals following the operation.

The Inulin Clearance of Nonoperated Control Rats

From an inspection of Table I it is clear that the youngest rats have the greatest inulin clearance expressed in relation to body weight. These rats have a mean weight of 197 ± 3 g, and their mean inulin clearance is 1.10 ± 0.03 ml/min/100 g body weight. In all older groups of rats the inulin clearances are less in relation to body weight and range from 0.79 ± 0.07 to 0.94 ± 0.08

ml/min/100 g body weight. There is no significant difference between the inulin clearances relative to body weight of these latter five groups, but the inulin clearance of the first group is significantly different from the latter five. Thus the absolute inulin clearance of the rat is directly related to its body weight after the animal has reached about 300 g weight.

TABLE I

The Mean Inulin Clearances of Normal, Sham-Operated Control and Partially Nephrectomized Rats at Intervals Following Operation

Relation of clearance measurement to the time of operation	Control unoperated			Control sham operation			Partially nephrectomized 5/6		
	Mean weight	C inulin	C Inulin	Mean weight	C inulin	C inulin	Mean weight	C inulin	C inulin
	g	*ml/min*	*ml/min/100 g*	*g*	*ml/min*	*ml/min/100 g*	*g*	*ml/min*	*ml/min/100 g*
Preoperative*	206 ± 4 n = 10	2.38‡ ±0.12	1.15 ±0.05	190 ± 4 n = 12	2.09 ±0.08	1.09 ±0.04	195 ± 3 n = 11	2.07 ±0.13	1.06 ±0.05
Postoperative									
4–5 wk	296 ± 10 n = 10	2.69 ±0.03	0.89 ±0.07	348 ± 8 n = 9	3.04 ±0.10	0.87 ±0.03	302 ± 13 n = 10	1.38 ±0.11	0.47 ±0.04
7–11 wk	383 ± 6 n = 7	3.34 ±0.14	0.94 ±0.08	418 ± 7 n = 11	3.11 ±0.14	0.75 ±0.04	353 ± 11 n = 10	1.74 ±0.18	0.49 ±0.04
12–14 wk	420 ± 5 n = 8	3.98 ±0.19	0.79 ±0.07	445 ± 13 n = 15	3.47 ±0.12	0.78 ±0.03	416 ± 9 n = 13	1.51 ±0.11	0.37 ±0.03
15–17 wk	490 ± 4 n = 7	4.22 ±0.16	0.86 ±0.04	495 ± 5 n = 13	3.70 ±0.19	0.75 ±0.04	444 ± 11 n = 13	2.02 ±0.17	0.40 ±0.04
27–30 wk	551 ± 10 n = 7	4.34 ±0.17	0.82 ±0.03	563 ± 39 n = 7	3.52 ±0.24	0.63 ±0.03	473 ± 7 n = 10	1.67 ±0.13	0.35 ±0.02
43–50 wk				648 ± 26 n = 9	3.59 ±0.19	0.56 ±0.05	460 ± 27 n = 7	1.12 ±0.30	0.21 ±0.05

n, number of animals.

* The mean inulin clearance values of all 33 rats before operation were 2.17 ± 0.06 ml/min and 1.10 ± 0.03 ml/min/100 g. The mean weight of all these rats was 197 ± 3 g.

‡ Means ± standard error of mean.

Inulin Clearance in Sham-Operated Control Rats

The inulin clearances of sham-operated controls are seen in the middle columns of Table I and in the left hand column of Table II; in the latter rats, PAH clearances were measured simultaneously. The sham operation per se has little effect on the inulin clearances at least during the first 12 to 14 wk, and no significant differences are discovered between the inulin clearances of the sham controls and the nonoperated controls until the animals have grown much older and heavier. The inulin clearance of the sham-operated controls is less than that of the nonoperated controls 27 to 30 wk after operation when the sham-operated rats have a mean weight of 563 ± 39 g as compared to 551 ± 10 g for the nonoperated controls. A group of 9 sham-operated controls which was maintained for approximately 46 to 50 wk after the operation shows an inulin clearance of 0.56 ml/min/100 g. Unfortunately no comparable group of nonoperated controls was examined. Thus there appears to be a decrease in the inulin clearance of sham-operated rats as compared to nonoperated controls

TABLE II

The Mean Inulin and PAH Clearances of Partially Nephrectomized and Sham-Operated Control Rats at Intervals following the Operation

Time of measurement	Sham-operated rats			Partially nephrectomized rats		
	Weight	C inulin	C PAH	Weight	C inulin	C PAH
	g	*ml/min/100 g*	*ml/min/100 g*	*g*	*ml/min/100 g*	*ml/min/100 g*
1 wk preoperative	192* ± 6 n = 18	1.27 ± 0.04	2.70 ± 0.4			
1 wk postoperative	248 ± 4 n = 9	0.96 ± 0.05	2.72 ± 0.13	226 ± 5 n = 9	0.50 ± 0.02	1.14 ± 0.07
4 wk postoperative	343 ± 8 n = 9	0.94 ± 0.05	2.76 ± 0.20	316 ± 5 n = 9	0.41 ± 0.03	1.24 ± 0.11
8 wk postoperative	375 ± 13 n = 9	0.89 ± 0.04	2.28 ± 0.13	399 ± 6 n = 9	0.40 ± 0.02	1.22 ± 0.09
12 wk postoperative	450 ± 17 n = 9	0.78 ± 0.02	2.30 ± 0.15	403 ± 17 n = 9	0.34 ± 0.02	1.09 ± 0.05
24 wk postoperative	513 ± 17 n = 9	0.65 ± 0.03	1.74 ± 0.13	488 ± 16 n = 9	0.29 ± 0.02	1.10 ± 0.06

n, number of animals.
* Means ± standard error of mean.

which becomes clearly evident about 24 to 30 wk after the operation. A similar effect is also noted in the second part of the study as shown in Table II.

Inulin Clearance of Partially Nephrectomized Rats

As one would expect, partial nephrectomy greatly decreases the inulin clearance of the rats. This effect is readily seen in Tables I and II. In the first part of the study (Table I) the inulin clearance of the partially nephrectomized group is 0.47 ± 0.04 ml/min/100 g 4 to 5 wk after operation, while that of the nonoperated control group is 0.89 ± 0.07 ml/min/100 g at that time, and the inulin clearance of the sham-operated group is 0.87 ± 0.03 ml/min/100 g body weight. The effect of the operation is not so great as might be expected considering the amount of tissue removed. This is clear when inulin clearance values of partially nephrectomized rats 4 to 5 and 7 to 11 wk after operation are compared with inulin clearance values in the sham-operated controls of the same period. At both times the inulin clearance values of the partially nephrectomized animals are slightly greater than 50% of those found in the corresponding controls. Similar results are seen in the second part of the study (see Table II).

The inulin clearance of the partially nephrectomized rat does not remain steady as time goes on following the operation but shows a progressive decline in value. However, in the first part of the work (Table I) until 30 wk after operation it remains at least 50% of the corresponding value in the sham-operated controls. The partially nephrectomized rats which were studied between 43 and 50 wk after the operation show a mean clearance of 0.21 ± 0.05 ml/min/100 g as compared to 0.56 ± 0.05 ml/min/100 g in the sham-operated controls. At this time the value for the inulin clearance in the experimental animal is much less than 50% that of the sham controls. A progressive decline in inulin clearance is seen in the other group of partially nephrectomized rats, but the process appears earlier (see Table II). The decrease in the inulin clearance of the partially nephrectomized rat exceeds the slow decrease in the inulin clearance of the sham-operated control. This was found also in the second part of the experiment (Table II) where inulin measurements were made in all the rats at each specified interval following the operation.

Validity of the Technique Used to Measure Clearance.—In order to validate the use of this technique for the measurement of clearance, it was necessary to be sure that the blood concentrations of the substances, the clearances of which were being determined, remain steady throughout the 90 min collection period. In preliminary experiments, we determined that our infusion technique did indeed maintain a steady blood level by sampling three or four times during the 90 min collection period. However, this was impossible in the clearance measurements on the experimental animals in the definitive study, for we could not draw too much blood for fear of interfering with hemodynamics and running the risk of changing the renal glomerular filtration rate (GFR) and blood flow. However, comparison of the concentrations of the inulin and PAH at the beginning and at the end of the collection period allowed a check on the steadiness during the clearance period. Except in three of our clearance determinations which were not included in the data presented, the plasma inulin concentrations at the beginning and at the end of the collection period differed by no more than 1 to 1.5 mg% from one another, and these clearance determinations were discarded. The range of plasma inulin level with which we worked was from 40 to 60 mg%.

Rate of Entry of PAH into Red Cells.—The rate of entry of PAH into red cells in the male rat may be seen in Table III. Also in the table is shown the amount of PAH added to each aliquot of blood, the hematocrit, and the plasma

PAH values at 5, 15, 30 min, 1 and 17 hr after adding the PAH. It is noted that while PAH enters the rat's red cell it does so slowly, and less than 10% of the serum PAH diffused into the red cells 30 min after the addition to the mixture. Even 17 hr after the addition of PAH but little more of it enters the cells than entered at 1 hr.

PAH Clearance in Sham-Operated and Partially Nephrectomized Rats.—The clearance of PAH is shown for a group of partially nephrectomized and a group of sham-operated control rats before and at intervals following operation (Table II). Extraction ratios for PAH were not determined in any of these particular animals, and the results are not corrected for extraction ratios which differ from 1.0. There is no early change in PAH clearance in the sham control rats

TABLE III

The Concentration of PAH in the Serum of Heparinized Blood at Intervals after the Addition of a Known Amount of PAH

Amount of PAH added to 10 ml blood	Hematocrit	Estimated concentration PAH in serum	PAH plasma concentration				
			Time intervals following addition of PAH				
			5 min	15 min	30 min	1 hr	17 hr
mg	*%*	*mg/100*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
0.10	42	1.73	1.72	1.65	1.55	1.51	1.50
0.10	40	1.69	1.70	1.65	1.58	1.51	1.50
0.10	43	1.76	1.75	1.66	1.61	1.54	1.55
0.10	42	1.73	1.70	1.63	1.57	1.53	1.50

following the operation. However, 8 wk after operation a fall in the clearance of PAH relative to body weight is seen which becomes striking 24 wk after the operation. In the partially nephrectomized rats there is a greatly reduced PAH clearance 1 wk after the operation, but no further change in the PAH clearance takes place during the following 24 wk. The mean PAH clearance ranges from 1.09 ± 0.05 ml/min/100 g to 1.24 ± 0.11 ml/min/100 g body weight at the five different times of measurements in this period.

The Extraction Ratio of PAH.—The values for the extraction ratio of PAH by the kidneys of nonoperated controls, sham-operated controls, and partially nephrectomized rats are presented in Table IV. The extraction ratio of non-operated controls ranges from 0.90 to 0.96 with a mean of 0.93. The values for extraction ratio of PAH in sham control rats are not significantly different from the nonoperated; they range from 0.89 to 0.97 with a mean of 0.94. In the partially nephrectomized rats, however, the extraction ratio is decreased, ranging from 0.58 to 0.82 with a mean of 0.71.

Glomerular Counts.—The values for the glomerular counts of nonoperated control rats, sham-operated control, and the partially nephrectomized rats are set out in Table V. The inulin clearance value for each rat obtained a day or two before the animal was killed for glomerular counting is also shown, and

TABLE IV

The Extraction Ratios for PAH of Partially Nephrectomized Rats, Sham-Operated Controls, and Unoperated Controls

	Weight of rat	Serum PAH concentrations		Extraction ratio (A − V)/A
		Arterial (A)	Venous (V)	
	gm	*mg/100 ml*	*mg/100 ml*	
Control Rats	431	0.32	0.02	0.94
	463	0.58	0.06	0.90
	430	1.32	0.06	0.96
	429	2.01	0.17	0.92
	492	0.68	0.06	0.91
Mean				0.03
Sham-operated controls	707*	0.36	0.04	0.89
	493*	1.02	0.01	0.99
	452*	0.98	0.02	0.97
	605*	0.70	0.08	0.89
	470*	0.84	0.02	0.97
Mean				0.94
Partially nephrectomized rats	530*	0.97	0.40	0.58
	496*	1.10	0.44	0.60
	267‡	1.17	0.35	0.70
	324‡	1.05	0.21	0.80
	321‡	1.24	0.32	0.74
	269‡	0.93	0.17	0.82
Mean				0.71

* Inulin and PAH clearances were measured on six occasions on these animals before the extraction ratio was determined. No such studies were made in the nonoperated controls.

‡ These animals were prepared for the purpose of this study, and the extraction ratios were measured between 4 and 5 wk after the operation.

in the right hand column the mean GFR per nephron is given. It is seen that in the partially nephrectomized rats the rate of filtration of some of their individual glomeruli must be very much greater indeed than in normal rats.

Histologic Studies.—In none of the kidneys which were examined histologically did there appear any abnormality of the glomerulus or the tubules which we could attribute to the repeated infusions of inulin and PAH.

DISCUSSION

The reduction in inulin clearance which was found to follow partial nephrectomy in the rat is not an unexpected finding; it reflects, we believe, the decrease in the number of nephrons. We assume that the clearance of inulin measures the glomerular filtration rate, although it is admitted that the presence of interstitial fibrosis and tubular disorganization in the renal remnant of the partially nephrectomized rat leaves this assumption open to more serious question than is the case in the normal rat's kidney.

TABLE V

The Inulin Clearances of Normal, Sham-Operated Control, and Partially Nephrectomized Rats Together with the Number of Glomeruli in the Kidneys after the Clearance Measurement Was Made

The right hand column shows the mean inulin clearance per nephron in each rat.

	Weight	Inulin clearance (GFR)	Glomerular count	Mean inulin clearance per nephron
	gm	*ml/min/rat*		*μl/min*
Normal rats	568	4.85	56250	0.086
	511	3.88	57000	0.068
	435	3.04	57500	0.053
	416	3.55	56500	0.063
Sham-operated control rats	515	3.38	53500	0.063
	628	3.62	52250	0.069
	414	3.19	48750	0.065
	406	3.40	44750	0.076
Partially nephrectomized rats	518	1.15	7500	0.153
	447	1.63	7250	0.224
	391	1.25	8000	0.156
	437	1.88	8000	0.235
	526	1.62	7500	0.216
	429	1.31	7750	0.169

The values for the glomerular filtration rate after partial nephrectomy represent more than 50% of values obtained at the same time in sham-operated controls, although the amount of renal tissue removed was considerably greater. This finding bears out the experience of Platt and his associates (11) who found the creatinine clearance of partially nephrectomized rats reduced less than the renal mass. Since renal mass in a partially nephrectomized rat increases following the operation as a result of the hypertrophy of those nephrons which remain, the extent of the adaptive change in the glomerular filtration rate of partially nephrectomized rats' kidneys is greatly obscured if it be related to renal mass alone. The glomerular counts which are presented here give a more satisfactory picture of the amount of functional renal tissue remaining than does the renal mass, and when the glomerular filtration rate is expressed in relation to the glomerular count, it is very clear that a much larger compensatory increase of glomerular filtration occurs in many of the remaining glomeruli than is apparent from a superficial consideration of the absolute filtration rate of the renal remnant as a whole. Expressed in other terms, the glomerular filtration rate per nephron is more than twice as great in the partially nephrectomized rat as in the control.

This conclusion rests on the assumption that all glomeruli in the normal kidney are functioning at any given time. Were this not so, inulin clearance in the normal rat might represent the glomerular filtration rate of only a fraction of the total nephron population. If the inulin clearance of the partially nephrectomized rats represents the glomerular filtration rate of all the nephrons of the renal remnant, no differences between glomerular filtration rates of the individual nephrons of normal and partially nephrectomized rats need be postulated to explain the data presented. Criticism of this kind is hard to meet, for although most studies purport to show that glomerular intermittency does not occur in dogs, rabbits, and other species (18–20), its occurrence in frogs has been directly observed (21). Moreover, a few investigators claim on the basis of their studies that it can indeed occur in dogs and rabbits (22, 23).

Were one to postulate glomerular intermittency to account for the data in this study, it would mean that only one third of glomeruli are filtering at any given time in the normal rat. Should this be the case, one would expect that glomerular intermittency could be readily recognized during the examination of the kidney in the living animal. No reports of such a phenomenon have appeared; indeed Walker and Oliver (24) specifically state they could find no evidence of it in rats when they made direct observations of the passage of oil droplets along the course of the renal tubules.

Moreover, our figures relating to the GFR of the sham-operated control rats are further evidence against glomerular intermittency in the rat. Were glomerular intermittency of any significant degree to occur in the normal rat, then a reduction in the number of glomeruli of the modest amount found in our sham-operated controls would be hardly expected to lead to the fall in glomerular filtration rate which we observed. This suggests that if any glomerular intermittency whatsoever be present in the rat, it is likely that the number of nonfunctioning glomeruli at any given moment under the conditions of our experiment must be less than 10% of the total number of glomeruli present. The fact that the inulin clearances in this study were carried out at very high rates of urinary flow further strengthens our contention that the data are not the result of changes in the degree of glomerular intermittency between the controls and the partially nephrectomized rats.

The decrease in the inulin clearance of the partially nephrectomized rat which occurs with time may be the result of a progressive obliterative lesion of remaining glomeruli. This is in accord with histological findings in the kidneys of partially nephrectomized rats killed 6 to 9 months after operation; in these kidneys many glomeruli show some fibrosis and obliteration of capillaries (12). The sham-operated controls also show a decrease of glomerular filtration rate over a long period of time, and here again there is the possibility that a glomerular sclerosis, particularly of the outermost glomeruli in the cortex, takes place. This kind of sclerosis might be brought about by removal of the capsule during the sham operation by interfering with the integrity of some of the outermost glomeruli of the cortex. The absence of a progressive decline in the glomerular filtration rate of the nonoperated controls with aging, at least over the period of time our study was made, strengthens the idea that some glomeruli may disappear in the sham controls as a result of the operation. This explanation of the findings is supported by the fact that glomerular counts made on the kidneys of sham control rats were significantly lower than those made on the kidneys of nonoperated controls.

The changes in glomeruli of the rat with aging were investigated by Moore and Hellman (25) who found glomeruli began to decrease in number after the rat reached 230 g weight. They observed that unilateral nephrectomy had no effect on the process. Their studies, however, were confined to counting glomeruli and did not include measurement of glomerular filtration rate. It appears from our observations that the progressive decrease in the number of glomeruli in the normal rat is not accompanied by any progressive decrease in glomerular filtration rate. This may be related to the fact that as glomeruli decrease in number with aging of the rat, they increase in size. Information concerning glomerular filtration rate relative to body size in the very old rat would be of interest, for Shock (26) has shown that in man glomerular filtration rate decreases with aging although, just as in the rat, a slow increase in glomerular size takes place (27).

We are unable to account for the abrupt change in the inulin clearance with respect to body weight of normal rats when they reach about 200 to 250 g weight. We are unaware of any sharp change in the proportion of body weight occupied by lean body mass or any modification of body composition in the rat at this stage of growth to which the variation in inulin clearance might be attributed. It appears likely to represent a true change in glomerular filtration rate rather than an alteration in the nature of the rat's growth. This observation, however, serves to emphasize the difficulty of interpreting those GFR values for rats which are given in the literature where the weight of the rats used is not recorded, and the GFR relative to body weight is the only figure quoted. This may account in part for the wide variation in GFR values in the rat reported by different workers.

Interpretation of PAH clearance data is on less solid ground than that of inulin clearances. The effect of partial nephrectomy on the regulatory mechanisms concerned with blood flow is unknown. Moreover, it was shown that discrepancies between blood flow determined by direct means and by PAH clearance results in part from failure to consider lymph flow in clearance studies (28). To what extent changes in lymph flow may affect PAH clearances in surgically damaged kidneys is unknown. Apart from these considerations, there is a decrease in the tubular extraction of PAH in the partially nephrectomized rat. This change in the tubular extraction of PAH may result from perfusion of tissue which fulfills no excretory function, or it may be caused by a relative failure of the transport mechanism in the remaining nephrons of the partially nephrectomized rat. This may result from an innate alteration in tubular function, but there remains the possibility that a greater load of PAH is being presented to functionally normal nephrons than they can transport; in this way, the tubular mechanism of the nephrons would be overtaxed. This latter explanation is suggested by the observation that if one takes into account the decreased extraction rate in the partially nephrectomized rats, the reduction in plasma flow in them is only 30 to 40%, while the nephron population is decreased by $\frac{5}{6}$ and thus a greatly increased plasma flow per nephron might be expected.

Nevertheless, it is interesting that PAH clearances of the partially nephrectomized rats show no change relative to body weight even 24 wk after operation, despite the progressive decline of the GFR. This evident difference in the progress of the GFR and the PAH clearance in the partially nephrectomized rat leads to a progressive fall in filtration fraction in these animals following partial nephrectomy. It is difficult to escape the conclusion that there takes place a progressive impairment of glomerular filtration in these animals unattended by a parallel change in tubular function, certainly with respect to the transport of PAH. If PAH clearance does measure effective renal blood flow in these animals, either filtration takes place into some nephrons which are blocked and do not contribute to the final urine, or a redistribution of blood flow in the renal remnant occurs with less perfusing the glomeruli, while the tubules are satisfactorily perfused. A redistribution of blood flow of this kind might be expected to accompany the progressive fibrosis of glomeruli which takes place in the partially nephrectomized rat, and which could lead to shunting of blood around the glomeruli through new anastomotic pathways. If shunting of this kind entails a compensatory increase in the medullary blood flow, then the urinary concentrating defect which the partially nephrectomized rat has been shown to have might also be expected.

Those factors which are responsible for the compensatory hypertrophy of the renal remnant after partial nephrectomy are unknown. It has been speculated that the reduction in renal mass increases demands on residual tissues, but the nature of this increased demand remains uncertain, and no specific process of renal function can be singled out as a prime factor in stimulating growth. The increased glomerular filtration rate per nephron following partial nephrectomy, which is shown here to occur, would be expected to lead to tubular dilation, and this in turn might stimulate hyperplasia of the tubular cells. The increased glomerular filtration rate also leads to an increased load of fluid and solute presented to the tubular cells for transport; since this entails increased tubular cell activity one might expect resultant hyperplasia. However, the nature of the stimulus for the increased glomerular filtration rate per nephron itself is quite uncertain. Recently Kleiman, Radford, and Torelli (29) claimed that in the rat the GFR correlates with the level of urea in the blood. If this be so, one would certainly expect the GFR per nephron to be higher in the partially nephrectomized rat than in the normal rat, for it is well established that the BUN of partially nephrectomized rats is raised from the time of operation.

SUMMARY

A method is described for the measurement of inulin and PAH clearances in rats without killing the animal. Inulin clearance measurements of partially nephrectomized rats and sham-control rats were made before operation and at intervals following the operation; inulin clearances were determined on normal rats at intervals during their growth. In another series of partially nephrectomized rats and their sham-operated controls, inulin and PAH clearances were determined in all the animals before and at intervals following the operation. Glomerular counts were made in some rats.

After partial nephrectomy the inulin clearance is reduced but not as much as would be expected considering the amount of renal tissue removed. The mean inulin clearance per nephron is greatly increased in the partially nephrectomized rat when compared to the value determined for the control rat. The inulin clearance of the partially nephrectomized rat shows a progressive decline which is first clearly evident about 6 months after operation. The sham-operated rats showed an inulin clearance slightly less than that of nonoperated controls about 24 to 30 wk after the operation. In the normal rats the inulin clearance relative to body weight is much greater in rats with a mean weight of 197 $\pm$ 3 g than in normals which are older and heavier.

The PAH clearance of the partially nephrectomized rat is reduced following operation but undergoes no further change in the ensuing 24 wk.

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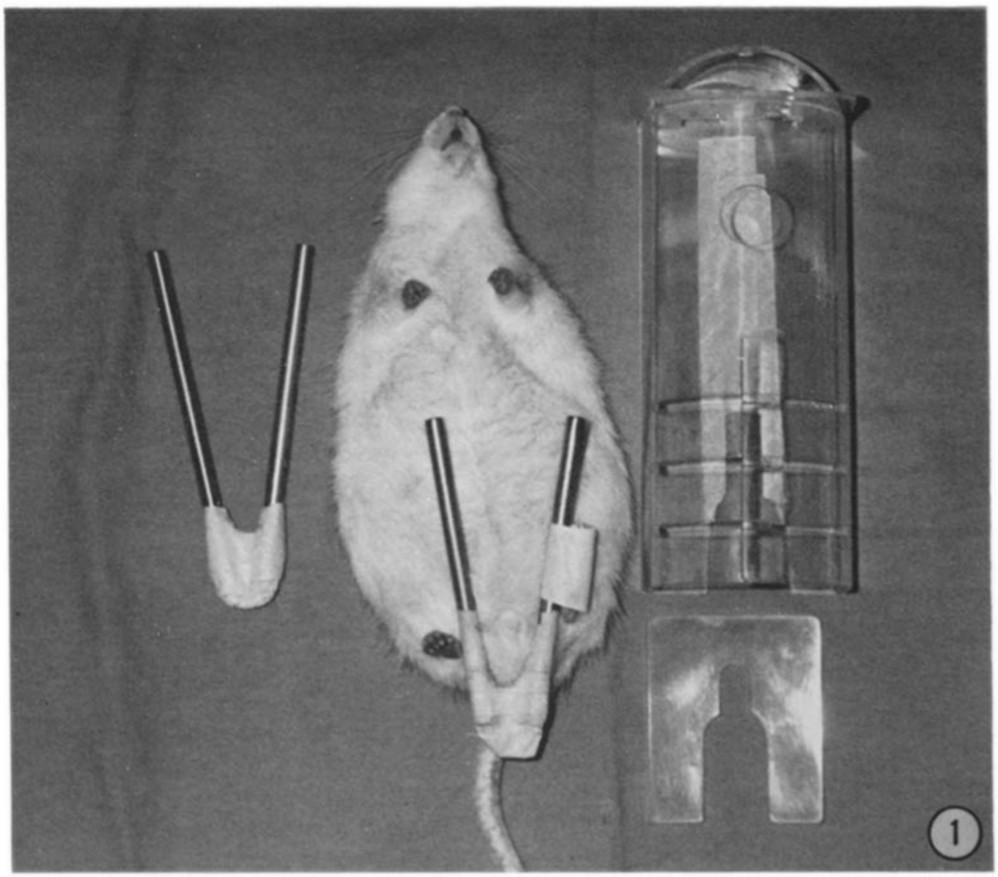
EXPLANATION OF PLATE 80

FIG. 1. This shows the V shaped support used to immobilize the rat. It was made by inserting a semirigid wire into the bore of two 6 in. long, stainless steel tubes ordinarily used in water bottles for animals. The wire was taped with masking tape at the vertex of the formed V so that a single unit resulted. The outside width at the open end is 2¼ in. when used with a regular size immobilization cage, and 3 in. when larger size cages are used. The cages were of clear acrylic plastic in regular and large sizes, purchased from Scientific Products, Division of American Hospital Supply Corp., Flushing, New York. They were modified by widening the rear inch of the bottom slot of the cage to between 1 and 1¼ in. and extending it through the rear of the cage to form a widened bottom slot of 1½ in. in length. Since male rats were used we found it advisable to widen the lower half of the tail gate to allow greater clearance for the testicles which project posteriorly as the rat rests in the cage. The restraining cage is shown on the right with the slot in its base through which urine collections are made. The rear gate of the cage is shown lying behind it and shows the lower half of the tail slot widened to allow greater clearance for the testicles of the male rats.

This picture also shows how the V-shaped rack was positioned. This was done by fitting the rack to the animal so that the body lay within the arms of the V, the apex of which pointed posteriorly. The hind feet and legs were astride the arms of the V, and the tail extended under the vertex of the V. To minimize subsequent discomfort which could lead to struggling during the clearance determination, the hind feet and legs were placed in as nearly normal a weight-bearing position as possible. Thus each hind leg was taped with the sole of the foot in the horizontal plane through the uppermost line of the respective arm of the rack by encircling the foot and tube with masking tape.



(Morrison and Howard: Clearances in partially nephrectomized rats)