OBSERVATIONS ON THE NATURE OF THE ENLARGEMENT, THE REGENERATION OF THE NERVES, AND THE FUNCTION OF THE CANINE RENAL AUTOGRAFT*

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CANINE renal autografts were observed for periods up to 12 months to determine : the nature of the enlargement of the graft, a usual sequela when the autograft is an only kidney ; the time of appearance and time required for the regeneration of nerves ; function of the autograft, particularly as it might be related to enlargement of the graft and the regeneration of its nerves. Quantitation of the factors in enlargement of the graft included an initial weighing and linear measuring, and needle biopsy. Neural regeneration was studied by microscopic examination of the nerves on the graft side of the anastomoses after staining of the tissues by the Bodian (1936) method. Function of the renal graft was tested by the response to water-loading and water-deprivation, urine analysis, determination of phenolsulphonphthalein excretion, blood urea nitrogen levels and the arterial blood pressure. From previous experience (Bricker, Straffon, Mahoney and Merrill, 1958 ; Murray, Lang, Miller and Dammin, 1956), it was expected that no significant compromise of renal function would occur.

MATERIALS AND METHODS

Randomly selected healthy female mongrel dogs weighing 12–16 kg. were used and were divided into the following 3 groups.

Group 1 (*Delayed autografting of the solitary kidney*).—Three animals comprised this group. The initial operation was nephrectomy, and 3 months later the remaining kidney was transplanted to the iliac vessels.

Group 2 (*Simultaneous nephrectomy and autograft*).—Four animals. At the initial operation, unilateral nephrectomy was performed, and the remaining kidney was autografted.

Group 3 (*Nephrectomy and pedicle clamping*).—Four animals. At the first operation one kidney was removed ; the pedicle of the remaining kidney was clamped for 1 hr.

Operative techniques

Autotransplantation to the iliac fossa (Murray *et al.*, 1956). After anaesthetizing the animal with intravenous sodium pentobarbital (30 mg./kg.), a midline abdominal incision was made, extending from xiphoid to pubis. The common iliac artery and vein were dissected for a distance of about 3 cm. from the aortic and inferior vena caval bifurcations. After clearing all the areolar adventitial tissue from these vessels, bulldog clamps were placed at their origins and the vessels were divided slightly proximal to the internal iliac origins.

The contralateral kidney was dissected after an incision had been made in the peritoneal reflection leaving an envelope of peritoneum on the anterior two thirds of the renal surface. The renal vessels were then isolated, cleared of areolar tissue and nerves, and ligated and

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divided as closely as possible to their origins. The ureter was transected approximately 5 cm. from the renal hilum and the distal segment was ligated.

The kidney was weighed measured in its longitudinal and lateral axes and biopsied by piercing the cortex with a hollow needle (internal diameter : 3 mm.) using a rotatory motion, thereby obtaining a cylindrical plug. Bleeding from such biopsy sites was usually arrested by a single stitch.

The kidney was then put into the iliac fossa and the anastomosis begun. The arterial anastomosis was achieved by the continuous, everting method of Carrel (1908, 1911) ; the venous anastomosis was executed with a continuous. " over-and-over " stitch. The suture material was 5–0 vascular silk on an atraumatic needle. The ureterovesical anastomosis was completed by embedding the " fish-mouthed " end of the ureter in the bladder lumen and securing it with 2 anchoring stitches. The kidney was fixed to the tissues lateral to the psoas muscle by means of stitches passed through the renal capsule and parietal peritoneum, and the midline abdominal wound was closed with chromic catgut and nylon retention sutures. The latter were removed on the 14th post-operative day.

During the procedure each animal received 300 ml. of 5 per cent glucose in water intravenously. The duration of total renal ischaemia never exceeded 1 hr.

Renal pedicle clamping.—The animal was anaesthetized and the abdominal cavity entered as in the autografting procedure. The renal vessels were isolated without stripping their nerves or areolar tissue. Bulldog clamps were applied to the artery vein and ureter for 1 hr. When no bleeding occurred after lightly pricking the organ's surface, it was assumed that complete ischaemia obtained. During this period of interrupted blood flow the opposite kidney was removed. A tissue specimen was taken from both kidneys, using the hollow needle. After the ischaemic period was completed, the abdomen was closed in the manner described above.

All animals received 300 ml. of 5 per cent glucose in water during the procedure.

No anticoagulants were used in either the autografting or pedicle clamping operations.

Measurement and biopsy

At monthly intervals concurrent with the tests of function, each animal was subjected to laparotomy. In order, the kidney was measured in its sagittal and transverse direction, and a hollow needle biopsy plug was obtained.

Studies of function

For 3 days prior to each operation each dog had a daily urine analysis, intake and output recording of fluid volume, and testing of phenolsulphonphthalein (PSP) excretion. For the latter, each animal was given a 300 ml. water load (by gastric tube), and PSP (1 ml.) was given intravenously 15–20 min. later. Aliquots of urine were obtained at 30, 60 and 90 min. after PSP injection. For testing response to water restriction, each animal was restricted to a fluid intake of 300 ml. during one of the three 24-hr. test periods.

Urine collection was by means of a No. 12 (French) Foley indwelling catheter, connected to a flexible plastic bag.

Blood urea nitrogen was determined in all dogs at the outset of the experiment, and at 1–2-monthly intervals thereafter. Immediately before each operation, prior to anaesthesia, arterial blood pressure was measured in each animal by means of direct femoral arterial puncture with a No. 18 needle connected to a mercury manometer.

Termination of the study

At the conclusion of each experiment, the animal was killed by nembutal overdosage, and the kidney was immediately removed. After the kidney was weighed and measured (before draining its contained blood), specimens of the hilar structures (in cross and sagittal section) and the renal parenchyma were obtained.

Histological studies

One half of each renal tissue specimen was immediately frozen in air, using ether-dry ice as the coolant ; the remaining half was fixed in 10 per cent formol-saline. The frozen tissues were sectioned, and stained with haematoxylin and eosin. The fixed tissues were embedded in paraffin, sectioned, and stained with haematoxylin and eosin and periodic acid-Schiff stain.

Using an American Optical Company micrometer, all of the glomeruli of each frozen specimen presenting a midsagittal plane were measured. That is to say, the entry point of the preglomerular arteriole and the exit point of the convoluted tubule were both visualized in the same glomerulus.

The remainder of the parenchyma was also examined, in the fixed specimens, with especial attention to inflammatory response, fibrosis, tubular configuration, and mitosis.

The renal hilar structures were fixed in 10 per cent formol-saline, and stained by the method of Bodian (1936). These were examined to determine the appearance of neural elements, vascular structures, and the ureter.

RESULTS

In the Table are summarized the data relating to hypertrophy (by weight) of the kidneys of groups 1 and 2. Two of the three kidneys of group 1 (wherein grafting was performed after the kidney had been in the solitary state for 3 months) demonstrated insignificant weight changes after grafting. The third kidney of group 1 was pyelonephritic. In contrast, the kidneys of group 2 (simultaneous nephrectomy and autograft) underwent hypertrophy of 21–55 per cent during the 9–12 month interval.

TABLE—*Comparison of Changes in Weight of Kidneys which were Autografted after Functioning as a Solitary Kidney (Group I) with Kidneys Autografted at the Time of Nephrectomy (Group II)*

Animal No.	Weight of kidney at time of autograft (g.)	Duration of graft (months)	Weight after autograft (g.)	Absolute change	Per cent change
Group 1					
22	61·9	11	96·7*	34·8	56·0
31	50·3	12	49·0	−1·3	−2·6
32	52·9	12	60·8	7·9	14·9
Group 2					
23	46·3	12	58·5	12·2	20·9
25	31·0	11	48·2	17·2	55·5
26	44·0	9	57·8	13·8	31·4
35	27·0	12	35·5	8·5	31·5

* This kidney developed pyelonephritis, which accounted for most, if not all, weight gain.

Studies of gross function

There were no significant or persistent changes in arterial blood pressure, blood urea nitrogen, phenolsulphonphthalein excretion, and the response to water loading and water deprivation. This applied in all groups, with the exception of the one animal in group 1 with severe pyelonephritis. In this case, the blood urea nitrogen rose to 125 mg./100 ml. shortly before death in the eleventh post-operative month.

Histological findings

An average of 4 glomeruli cut in the midsagittal plane were found in each tissue section. In all 3 groups of animals, the control and test glomerular diameters were remarkably constant, and in the 0·13–0·15 mm. range. Therefore, there was no detectable glomerular enlargement in any group.

Aberrations from normal histological patterns were inconstant and minimal. An occasional hyaline or epithelial cast and occasional focal pyelonephritic scars were found. In Figs. 1 and 2 are depicted photomicrographs of renal parenchyma before and after autografting.

Evidence for nerve regeneration was established by the presence of non-fragmented axis cylinders within nerve bundles cut in a longitudinal plane. Because of the thread-like character and circuitous route of the renal nerves travelling in the loose adventitia surrounding the hilar vessels, obtaining nerves cut in longitudinal section required several histological sections of each specimen. In some slides several nerve bundles could be found travelling short distances parallel to the plane of sectioning. It was in such segments that the condition of the axis cylinders was assessed.

From another experiment, it was learned that one week following autografting, the nerve fibre architecture of the hilar structures was completely destroyed and there was extensive dissolution and necrosis of Schwann cells and only a rare axis cylinder fragment could be identified (Fig. 3).

Three months after autografting, the ratio of axis cylinders to Schwann cells was reduced, due mainly to proliferation of the Schwann cell component. These cells were oriented parallel to the long axis of the nerves. Between many Schwann cells, non-fragmented axis cylinders were seen (Fig. 4). Because of the thickness of the tissue sections required for optimal silver impregnation (20 μ) these nerve cylinders could be more easily followed and appreciated under the microsope than in a photomicrograph of a single optical plane.

At 6 months after autografting, it was especially appreciated that nerve regeneration is a variable process. In some of these specimens there were significant degrees of fibrosis and chronic inflammation within the perivascular fibro-fatty tissue possibly related to surgical trauma and haematoma formation. Possibly purely local mechanical factors accounted for lack of regeneration. In other regions, however, there was evidence of nerve regeneration and a relatively normal nerve pattern was present (Figs. 5 and 6).

DISCUSSION

Innervation of the kidney

The importance of renal nerves in controlling renal function has long been in dispute. Since Holbrook's early anatomical study (1883), several investigators have elucidated the nerve elements found throughout the kidney and its related structures (Gruber, 1933 ; Mitchell, 1951).

The genito-urinary system is innervated by fibres from spinal cord segments T. 4 to L. 5. The renal plexus itself is fed by the semilunar ganglion, which communicates with the greater and lesser splanchnic nerves, and with the vagus nerves. The majority of these neurons derive from the sympathetic system, which provides both vasoconstrictor and vasodilator effects. It is well established that stimulation of the peripheral end of the divided splanchnic nerve results in renal contraction, presumably due to vasoconstriction. However, Bradford (1889) in an early study in dogs wherein the spinal roots were stimulated, found that the effect on renal vasculature depended on the rate of stimulation : quick rates (10–50 per sec) produced vasoconstriction, and slow rates (1 per 1–2 sec) produced vasodilatation.

It has been concluded that the majority of renal nerves are postganglionic, since very few ganglion cells have been found in the hilus or parenchyma. Free nerve endings have been identified in the adventitia of the small blood vessels, the glomeruli, and in the tubules (Harman and Davies, 1948 ; Mitchell, 1951).

The ureters are innervated from the renal, spermatic (or ovarian), and hypogastric plexuses. These nerves follow the small arteries of the ureteral adventitia (" Grund-plexus " of Engelmann), distributing many fibres to the tunica muscularis and a few fibres to the mucosa. Both medullated and non-medullated fibres have been found. A few ganglion cells appear throughout the entire length of the ureter, but the majority occur in the lowermost 3–4 cm. Ureteral peristalsis, however, is known to be primarily dependent on intrinsic activity of the smooth muscle ; it can function quite independently of nerve supply.

Quinby (1916) was one of several earlier workers who demonstrated that total renal denervation, by renal autografting, does not significantly impair function. Numerous, more recent investigations purport to demonstrate that varying degrees of increased water and sodium excretion result from renal denervation by other methods, including excision of the splanchnic nerves, stripping of the renal vessels, and phenolization of the renal pedicle (Bricker *et al.*, 1958 ; Meilman and Winer, 1953 ; Page, Baxter, Reem, Scott-Baker and Smith, 1954 ; Surtshin and Schmandt 1956). Many of these studies show such wide variance in both control and test groups that interpretation is made difficult.

It is, of course, a certainty that nerve degeneration occurs after division of the renal pedicle, but no data are yet available defining the time of appearance of regeneration. Nor has the effect of moving the pedicle to the iliac vessels been evaluated, a situation in which cells responsible for regeneration may ultimately innervate a structure new to this site.

The presence of axis cylinders in portions of the renal nerves distal to the line of anastomosis suggests to us that a certain amount of neural regeneration had taken place as early as 3 months. Re-proliferation and orderly longitudinal alignment of Schwann cells in itself implies a favourable environment for axis

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EXPLANATION OF PLATE

FIG. 1.—Needle biopsy specimen of kidney at the time of autografting. (Animal No. 35.) (H. and E.) ×64.

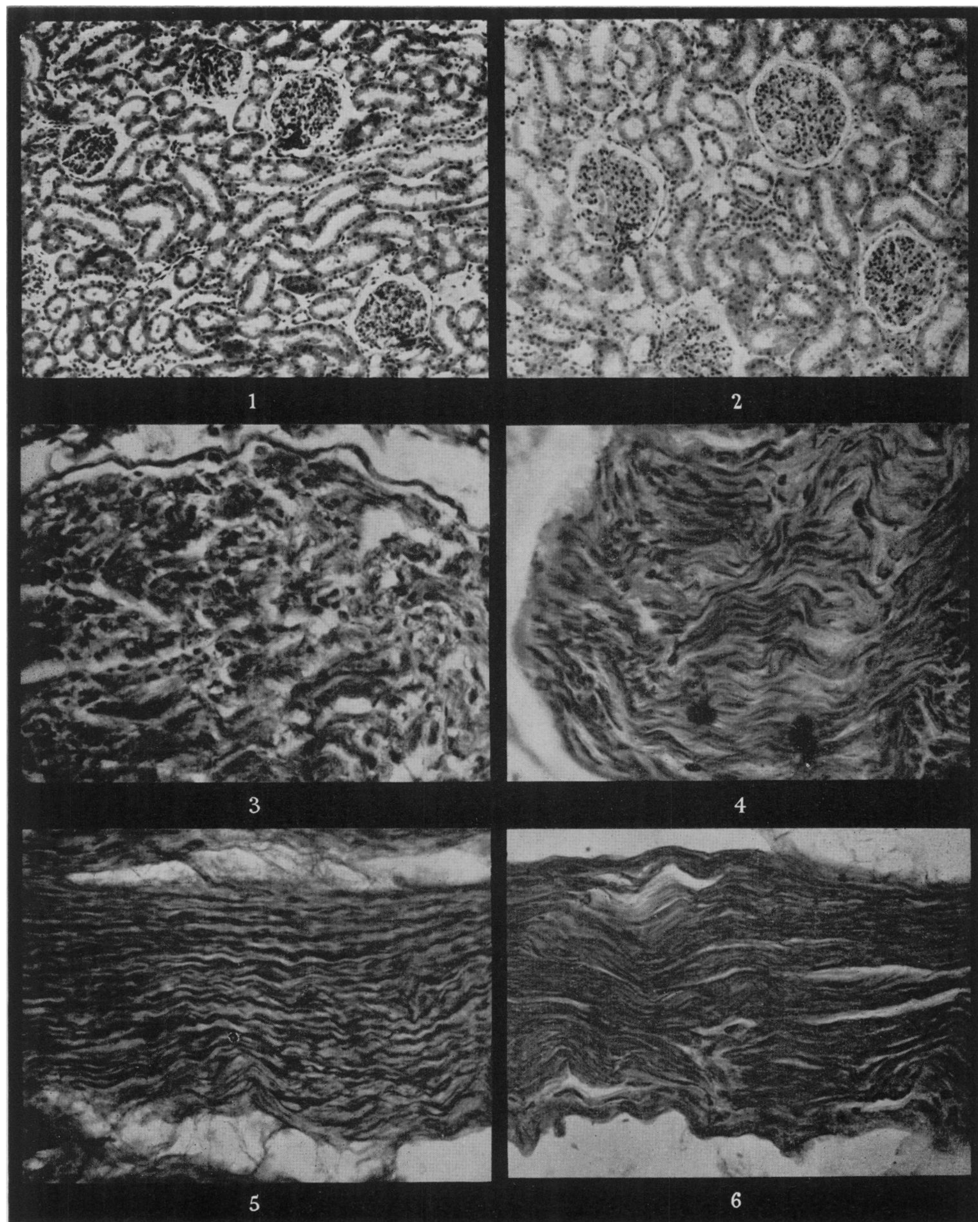
FIG. 2.—Histological appearance of the kidney shown in Fig. 1, 12 months following autografting. No obvious morphological change provides an explanation for the increase in weight. Although the interstitial tissue is more prominent and the tabular epithelium is slightly taller it is difficult to visualize how these account for the 31·5 per cent increase in weight. (Animal No. 35.) (H. and E.) ×64.

FIG. 3.—Sympathetic nerve distal to the line of vascular anastomosis one week following autografting. There is dissolution of nerve fibre architecture, fragmentation of axis cylinders and pyknosis of Schwann cell nuclei. (Bodian.) ×203.

FIG. 4.—Sympathetic nerve distal to the line of vascular anastomosis 3 months following autografting. There is early reorganization of nerve fibre architecture. Schwann cells are numerous and are oriented in parallel bundles. Axis cylinders are visible in some regions as thin filaments between Schwann cells. (Bodian.) ×203.

FIG. 5.—Sympathetic nerve distal to the line of vascular anastomosis 6 months after autografting. Neural architecture has been virtually reconstituted. Numerous wavy axis cylinders are present. Schwann cells are not as prominent as at 3 months but are more prominent than in a normal nerve. (Bodian.) ×203.

FIG. 6.—Sympathetic nerve lying in loose adventitia of the renal pedicle of a normal non-grafted canine kidney. (Bodian.) ×203.



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cylinder regrowth. These features are in marked contrast to the findings at one week, at which time complete necrosis of nerve bundles was present. Adverse local factors such as chronic inflammation and fibrosis possibly related to surgery and haematoma formation most probably account for the absence of axis cylinder regeneration in some of the 6-month specimens.

In an earlier study (Murray *et al.*, 1956), wherein only haematoxylin-eosin stained sections were used for the identification of nerves, regeneration was not recognized.

In a series of experiments in cats, Lee (1921) found that partially excised cervical sympathetic trunks regenerated fairly completely over an average interval of 35 days. The shortest interval was 26 days. Stimulation techniques as well as anatomical methods were used for estimating the degree of regeneration.

A better parameter for assessment of renal nerve regeneration might consist of studies designed to demonstrate functional variations in renal excretory mechanisms in the autografted specimen following stimulation of specific nerves.

Ischaemia

When its duration does not exceed 2 hr., occlusion of the renal artery has been found, in dogs, not to result in serious functional impairment (Hamilton, Phillips and Hiller, 1948). The animals of group 3 were used as controls pertinent to this factor. No differences, either functional or anatomical, were found to exist between these kidneys and the autografts. It is concluded that the denervation incidental to autografting is not followed by detectable histological change of the renal parenchyma.

Hypertrophy

Enlargement of the solitary kidney is a well known process occurring secondary to interruption of function in the contralateral kidney, whether by agenesis, resection, or ureteral ligation. The specific agents responsible have not been identified, but there is substantial evidence that they are humoral (Berglund, Medes, Huber, Longcope and Richards, 1935). It has been estimated that renal hypertrophy can occur in 20–30 days in man (Berglund *et al.*, 1935).

In the dog, compensatory hypertrophy is chiefly ascribable to enlargement (both lengthening and thickening) of the convoluted tubules ; although some investigators have described a slight increase of interstitial tissue. In the rat, the number of glomeruli does not increase (Arataki, 1926).

Hypertrophy would be measured more accurately by a volumetric technique which did not require excision of the organ. In this manner, sequential changes of size could be determined in any given kidney during any desired time interval. But, because the kidney is an irregularly shaped structure, and only 2 axes can be measured without mobilizing the organ, and because of probable variations in degree of renal vasoconstriction, our attempts at linear measurement yielded inconclusive data.

On the basis of gain in weight, the kidneys of group 2 (wherein autografting was performed simultaneously with nephrectomy of the opposite kidney) increased in size by 21–55 per cent somewhere before the end of the 9–12 month interval. In group 1 (wherein autografting was performed 3 months after removal of the opposite kidney), one of the grafted kidneys unfortunately developed pyelo-

nephritis. The remaining 2 kidneys showed slight changes in weight, which were well within the probable limits of error in the method.

These data indicate that, first, compensatory hypertrophy occurs after autografting if the contralateral kidney is removed at the time of the autograft, and, second, no significant further increase in size occurs if a kidney, previously solitary for 3 months, is autografted. It is justifiable to assume that hypertrophy had occurred in the latter type of kidney during the 3 month period prior to transplantation.

Additional parameters

The transplantation manoeuvre did not alter the mean arterial blood pressure. As other investigators have shown, the blood urea nitrogen, the rate of PSP excretion, and the responses to water loading and water deprivation were also not significantly affected. These functional studies were not intended as a precise physiological inquiry ; they were performed to provide a simple means of determining gross functional status of each kidney at any given time.

SUMMARY

Over periods of 9–12 months, 7 canine renal (iliac) autografts were studied in detail. Four additional animals were followed after renal-pedicle clamping for 1 hr., without denervation.

Anatomical evidence of nerve regeneration appeared in the five autografted specimens which were examined. Regeneration was found at 3 months, the first interval at which this parameter was studied. It appeared to be complete by 6 months.

When there was simultaneous nephrectomy and autografting, hypertrophy was observed, but, when autografting was delayed for 3 months after nephrectomy, no further increase in renal size occurred.

Sequential biopsies of renal parenchyma, obtained at monthly intervals, revealed no increase of glomerular diameter at any time. Moreover, there were no consistent changes in histological appearance of the parenchyma, with the exception of one dog in group 1, in which pyelonephritis developed.

This study, like others described, establishes the observation that the autograft as an only kidney is life-sustaining, shows no constant histological abnormalities, and has a normal function.

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