DISSECTIONS OF NEPHRONS FROM THE HUMAN KIDNEY

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MUCH of our exact knowledge of the structure, varieties and arrangement of the nephrons in the kidney of Man and of several lower animals is based on the monumental work of Peter (1909). He first applied and perfected for this purpose the method of maceration in strong hydrochloric acid which softens and removes the interstitial substance leaving the glomeruli and epithelial structures more or less intact. In this way he was able to separate large portions of many nephrons and occasional complete structures, and so to obtain measurements of the various segments and accurate data as to their positions and relationships in the kidney. He remarked on the great difficulty of securing permanent preparations of these delicate objects and on the relative rarity of obtaining suitably fresh human material for dissection. Huber (1911) later discussed certain modifications in the technique of maceration and applied the method with great success to kidneys of freshly killed rabbits, cats, dogs, guinea-pigs and rats. He first injected 75 per cent. hydrochloric acid into the renal arteries, then divided the organs into several portions and allowed maceration in strong acid to proceed for 3 or 4 hours. After subsequent washing in water the tissues were stained in bulk with Mayer's haemalum, washed, alkalinised, and finally submitted to dissection with very fine needles under the microscope. By this procedure Huber isolated a large number of complete nephrons which could be drawn to scale and measured, and several beautiful figures of these preparations are shown in his communications. Huber found difficulty in securing similar results from human kidneys, and in his well-known article on renal tubules (1932) he makes no reference to any later success with human material. Traut's (1923) studies of human kidneys were based upon specimens macerated in 50 per cent. hydrochloric acid for 8–12 hours, usually after the tubules had been injected from the ureteral aspect with ferrocyanide, so as to give the effect of a Berlin blue mass in the lumina: kidneys of foetus, children and adults were examined. His main conclusion was that the kidney could be subdivided into structural units composed of 140–180 nephrons which were connected, by successively uniting branches of collecting tubules, downwards to a single collecting duct of the eighth order of division: such units receive their blood supply from their periphery. He claimed also to have found it easy "to remove whole renal tubules or groups of two or three

still in their original positions and connected to the ducts", but he gives no measurements of any of the segments or of complete nephrons. As the dimensions of the single uriniferous tubules derived from each tuft appear to carry great significance in any attempt that may be made to realise the conditions under which urine is elaborated, much further work on this point is probably desirable. An opportunity to follow this up over a short period has recently presented itself, and the results obtained in semi-permanent mounting, measurement, and photographic representation of human nephrons may be deemed worthy of record.

TECHNIQUE

In attempting to obtain preparations of human tubules many valuable hints have been gathered from the works of Peter and of Huber, but the method of preliminary injection of arteries employed by the latter has not so far proved completely advantageous, though this may be owing to insufficient experience. The method which has proved most successful so far in our hands is as follows: A kidney as fresh as possible is cleared of fatty tissue, but the fibrous capsule is left intact. A half of the organ is placed in about three times its volume of 75 per cent. hydrochloric acid for 20 hours and then allowed to remain in 5 per cent. acid for 2 or 3 days. During this time the tissue swells and becomes somewhat transparent. The acid is then carefully washed out of the container by a stream of running water and the tissue allowed to remain in water for half an hour. Wedge-shaped portions of kidney, including cortex and corresponding medulla, are then cut off with the edge of a thin glass slide and transferred to a large Petri dish half-filled with fresh tap water. Dissection can be carried out at once or at any time in the following week. The addition of 0·5 per cent. formalin has been found to harden the tubules slightly and enable them to be kept and dissected even after a month. No staining of the tissues has been attempted. Dissection has been carried out with a single finely drawn out glass needle under a dissecting microscope at a magnification of $\times 10$. The process of dissection consists in the slow and careful following up of a single tubule with the glass needle and gently separating it bit by bit from the other tubules to which it may be lightly adherent. In working the needle point between adherent segments, breakages naturally occur very easily. The narrow limbs of the loop present especial difficulty, and particularly those embedded in the denser connective tissue of the medulla. It is impossible completely to straighten out the natural coils of the convoluted tubules. The attempt has generally been made to retain the natural adhesion between the upper end of the ascending limb of the tubule and its own glomerulus. Once a nephron is separated it is raised gently in the water with the needle and there is thrust under it a slide which has previously had a rectangular dam of vaseline applied on its upper surface. When the structure has settled down on the slide and been properly arranged by gentle movements with the needle, a suitable large cover-glass is lowered over it in the water and pressed down on the vaseline

until sealing is just completed. The whole preparation can then be removed from the water. The nephrons are thus enclosed in formolised water in a cell about 0·5 mm. in depth. Many of those mounted still remain in good preservation after six months.

Measurements can be carried out directly on the straight parts of the tubules by use of stage and eyepiece micrometers. In all instances, however, the preparations have also been photographed at magnifications of $\times 10$ to $\times 20$ and tracings made from prints of these with the aid of microscopic observations on the original slides. From the tracings, finally, the measurements of the various coiled segments have been obtained by following their midlines with a rotameter or map measurer and making allowance for the magnifications. As Huber has pointed out it is impossible to make an exact allowance for the upward and downward curves in pictures of the convoluted parts, and measurements of these will always be slightly underestimated.

The photography of the slightly brownish unstained tubules has been carried out with Winkel micro-luminars 35 and 50 mm., using Ilford process plates, backed, with a Wratten C filter (blue), to increase contrast.

ORIGINAL OBSERVATIONS

The cases from which dissections were obtained were as follows:

(1) Male, aet. 20 years (G.R.I. 15,385), died of acute meningitis at 3.45 a.m. on 3. v. 34. Post-mortem examination 7 hours later.

(2) Male, aet. 11 years (G.R.I. 15,426), died of tuberculous meningitis at 3.35 a.m. on 4. vi. 34. Post-mortem examination 6½ hours later.

(3) Male, aet. 10 years (R.H.S.C. 3944), died of acute enteritis at 7.35 a.m. on 13. vi. 34. Post-mortem examination 4 hours later.

The record of results falls into two parts, first a general account of the different types of nephron which corresponds in the main with the fuller descriptions by Peter, and secondly the measurements obtained from some complete or almost complete structures.

(*a*) The first type of nephron to be described starts from a glomerulus in the deep part of the cortex. In some kidneys these glomeruli are larger than those situated peripherally, though in the kidneys dissected by Peter this difference was not found. Starting from the tuft the first convoluted tubule, which appears greyish brown and granular, winds towards the periphery of the kidney in a closely packed mass, not mingling with other tubules. After several main coils with innumerable fine subsidiary turnings, it returns near to its own tuft and then extends almost straight towards the medulla in a ray, along with similar segments of other tubules. It gradually narrows at the edge of the cortex to be succeeded by the narrow limb of the loop. This is distinctively clear and transparent and extends for a variable but often considerable distance straight into the medulla, sometimes reaching nearly to the papilla. It then bends, forming the actual loop of Henle and returns straight towards the boundary zone, changing to broad ascending limb in a zone of

about 3 mm. width, short of the margin of the cortex. This broad limb is again granular and cloudy compared with the narrow one. It passes straight to the glomerulus of the same nephron (Plate I, fig. 1) and becomes fairly firmly attached to one of the arterioles, according to Peter, always the efferent vessel. From this point the tubule exhibits several sharp bends, forming the characteristic portion described by Peter as the "Zwischenstück". This short segment, which can also be regarded as the commencement of the distal or second convoluted tubule, is the only part of the latter which lies near the tuft. Thence it passes on as the second convoluted tubule in a zigzag course between masses of first convoluted tubules to join another structure of the same type before ending in a collecting tubule (Plate I, fig. 2). The opinion of Traut (1923, p. 117) that the distal convolutions lie in closest apposition to the glomerulus and that the proximal convolutions lie for the most part superimposed on those of the distal portion is quite contrary to our observations, as it is to the descriptions by Peter. Judging from our measurements and photographs, which reveal the preponderance in bulk of the proximal tubules in all instances, Traut's less complete macerations have prevented him from distinguishing the distal from the proximal segments. His conclusion also does not appear to be borne out by his fig. 12, which clearly shows the distal tubule ascending past the tuft towards the periphery. The cells in the Zwischenstück are the clearest in this part of the tubule, while the middle part of the second convoluted tubule is darkest and most granular. The collecting tubules further on are distinctively clear in appearance. While many of these deep nephrons have been traced, it has not been found possible so far to separate one intact owing to the difficulty of extracting the thin limbs from the dense connective tissue of the medulla.

(*b*) Nephrons of the second type arise from tufts in the middle or outer zone of the cortex. They differ from the first chiefly in that the narrow limb changes to the broad while still descending, so that the bend of the loop is formed by the latter (Plate II, fig. 1). The bends occur in the outermost zone of medulla or in the medullary ray in the cortex. The narrow limb is of very variable length and sometimes measures only a few microns (Plate II, fig. 2).

(*c*) In some parts of the cortex, usually opposite medullary rays, there occur groups of short nephrons which possess no narrow limb (Plate II, fig. 3). The straight descending part of the first convoluted tubule joins the broad limb directly. The loop of Henle formed by the latter often shows a spiral turn. The broad limbs are shorter than in (*a*) and (*b*), but the second convoluted tubules are distinct and long. Structures of this type occur in closely associated groups and are difficult to separate. They have not been identified in all the kidneys examined.

The second convoluted tubules of the peripheral nephrons (*b*) and (*c*) appear to change directly to collecting tubules, whereas those of the deep nephrons join together before entering these. Considerable segments of collecting tubules have been separated, but no complete ones. A large portion

is shown in Plate III, fig. 1, with a nephron attached. The larger ducts of Bellini appear to break down rapidly after death.

The measurements obtained from complete or almost complete nephrons and from complete segments of some others are given in Table I.

Table I. *Lengths of tubules in millimetres.*

Case	Tubule	1st con. tub.	Narrow limb	Broad limb	2nd con. tub.	Total length
		(*a*) Complete or almost complete nephrons.				
1	A	20·65	5·0	14·53	1·875*	42·055
	B	20·72	5·6	15·0	2·34*	43·66
	G	19·06	3·75	13·75	3·437	39·997
	H	14·14	None	6·09	4·21	24·44
	L	23·12	1·66	10·62	1·79*	37·19
2	A,*a*	7·285	3·12	7·5	1·875	19·78
	A,*b*	8·44	2·7	7·08	2·34	20·56
3	B	8·7	3·3	9·1	1·7*	22·8
	C	11·6	3·3	9·58	1·56*	26·04
	F	11·56	3·125	9·375	3·75	27·81
	G	11·25	5·3	10·625	3·1	30·275
		(*b*) Complete segments only.				
1	C	18·9	—	—	—	—
	D	21·718	7·187	17·65	—	—
	F	21·875	—	—	6·32	—
	F,*a*	—	—	—	3·43	—
	I	17·81	13·875	—	—	—
2	B	8·75	—	7·5	2·5	—
Peter's av. figures (adults) (p. 197)		14·0	2–10	9·0	4–6	30–38

* 2nd con. tub. not quite complete.

The measurements of length vary somewhat, but those obtained from case 1 are comparable in size and range of variation with those given for the secretory parts of the tubules by Peter. 1 H, which is relatively short, is of type (*c*) with no narrow limb. Tubules 1 D and I are of type (*a*), and show the great length of the narrow limb. Peter estimated the length of these long narrow limbs at 4·5–16 mm. with an average of 10 mm. The rest of the tubules are of type (*b*) with narrow limbs very variable, 1·66–5·6 mm.

In the younger subjects 2 and 3 the tubules are distinctly shorter than in the adult but show the same general conformation. A point of pathological interest noted in the dissections was that in two of the cases a few tubules contained blood and accordingly stood out as prominent objects (Plate III, fig. 2). The even distribution of the blood in these first convoluted tubules from the glomerulus or near it downwards was in keeping with the view commonly accepted that tubular haemorrhages arise from the tufts.

A few measurements of tubule diameters are given in Table II. These are much greater than corresponding measurements given by Peter and may indicate some swelling of the material, though the procedure followed in preparation has been similar to his.

One main effect of this work is to emphasise again the great length of the secretory parts of the renal tubules, often about 4 cm. in the adult, compared

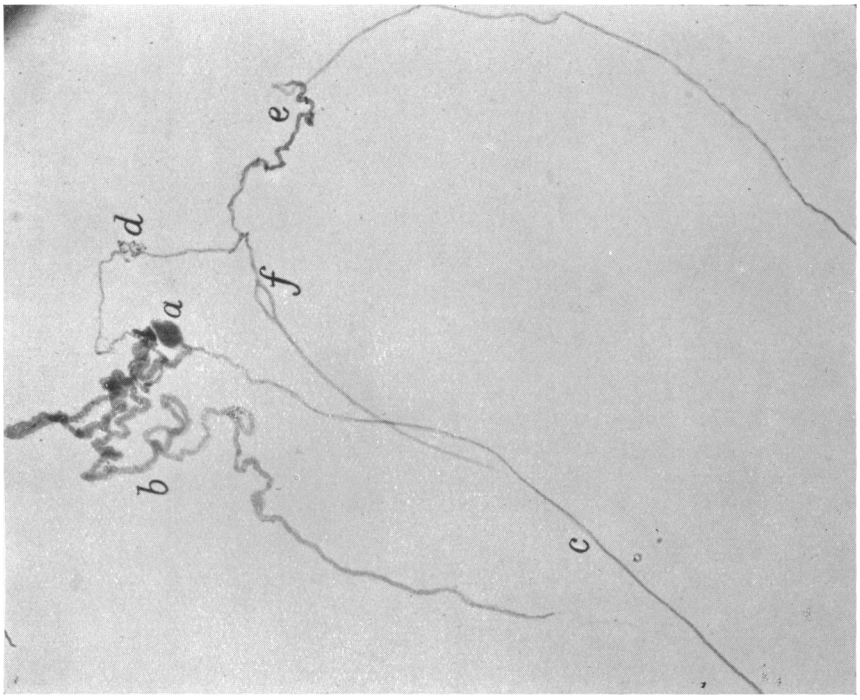
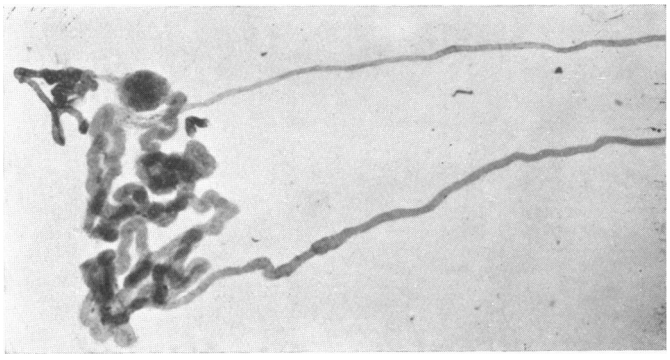


Fig. 2.



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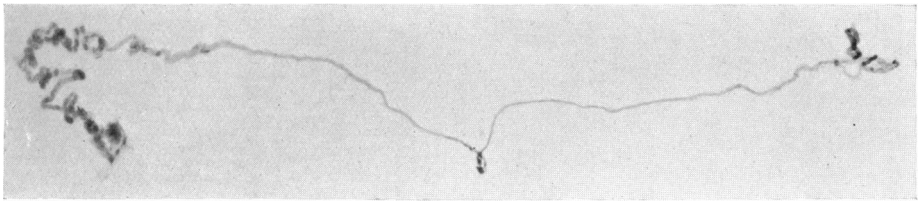

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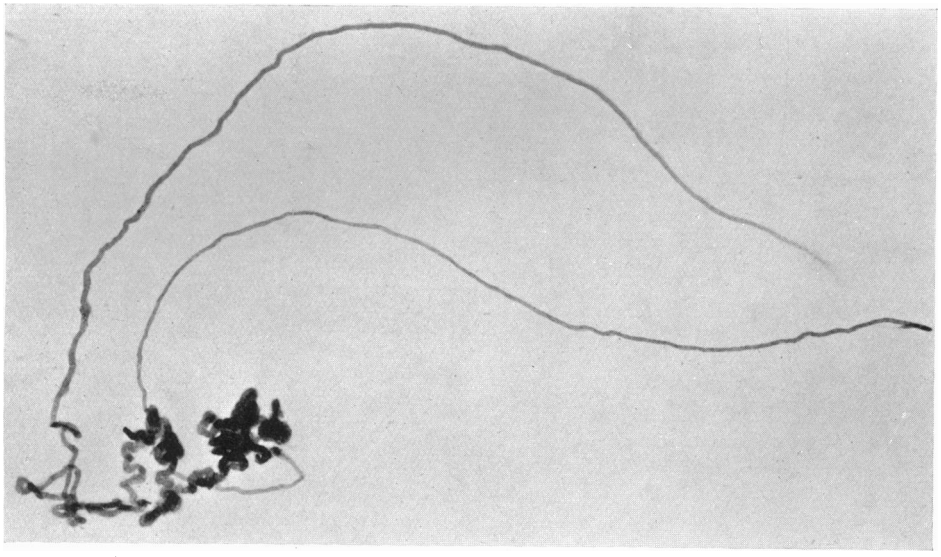

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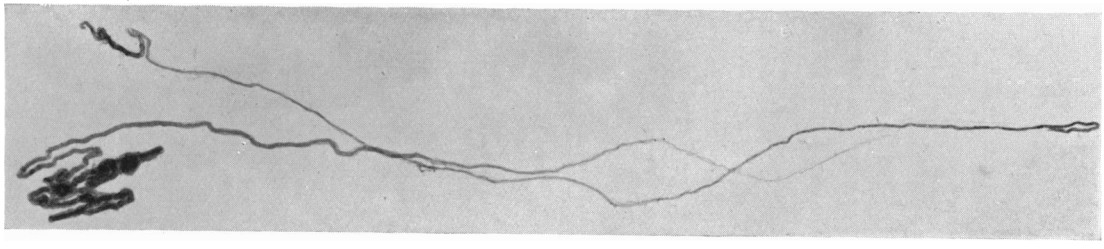

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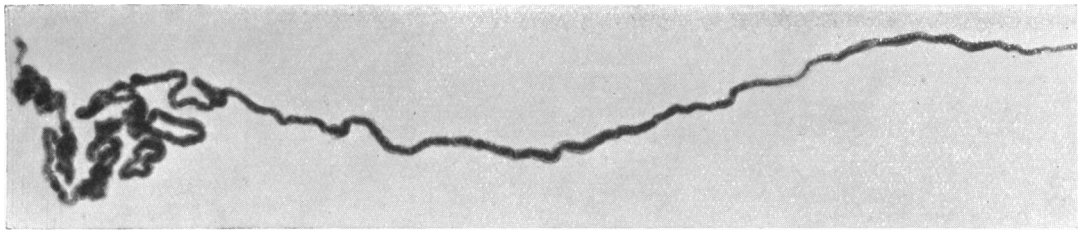

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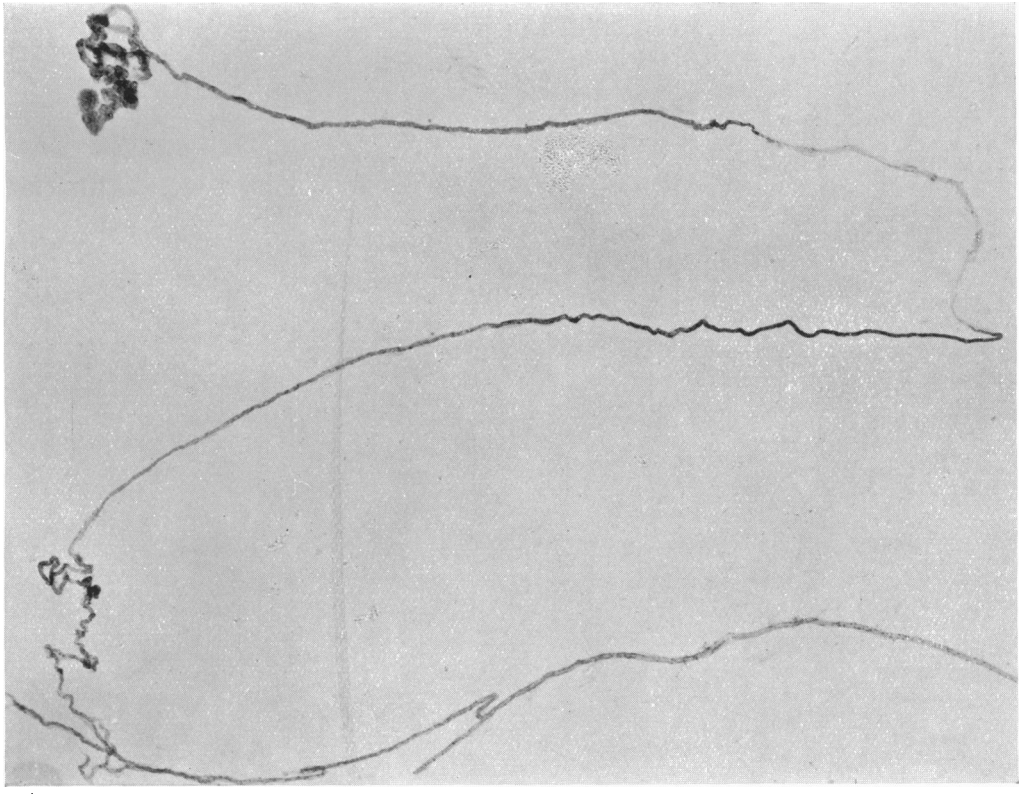

Fig. 1.

with their diameter, a feature which cannot fail to have significance in attempts to interpret renal functioning.

Table II. *Diameters of glomeruli and tubules in microns.*

Case	Tubule	Glomerulus	1st con. tub. Coiled part	1st con. tub. Straight part	Narrow limb	Broad limb	2nd con. tub.
1	A	300	79·7	72–51	30	45–51	60
	B	336–276	75·0	75–57	28	45–53	51
Peter's av. figures		152–159		57	15	30	41

In conclusion I express my indebtedness to the Management of Glasgow Royal Infirmary for laboratory accommodation, and to Prof. Shaw Dunn for valuable suggestions and assistance with photography.

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EXPLANATION OF PLATES I–III

PLATE I

Fig. 1. Peripheral part of a nephron in case 1. The first convoluted tubule is seen to start from the glomerulus on the right and after many windings to descend towards the medulla on the left. The broad ascending limb comes up on the right to the tuft, where it is attached, then passes above the latter as second convoluted tubule showing several zigzag bends. × 20.

Fig. 2. Junction of two second convoluted tubules of type (*a*) nephrons. The glomerulus *a* gives off first convoluted tubule *b* which sends down its descending part to end in a break of the narrow limb. The broad ascending limb *c* of the same nephron ascends from below and passes the glomerulus to form second convoluted tubule *d*. The latter joins a similar tubule *e* and the united tubule enters a collecting tubule *f*. × 10.

PLATE II

Fig. 1. Complete nephron (case 1 A) of type (*b*): shows a fairly long narrow limb, and the turn of the loop formed by broad limb. × 10.

Fig. 2. Complete nephron (case 1 L) with short narrow limb: type (*b*). × 10.

Fig. 3. Complete nephron (case 1 H) with no narrow limb: type (*c*). The broad ascending limb and second convoluted tubule have been drawn downwards. × 10.

PLATE III

Fig. 1. Complete nephron (case 3 F) attached to collecting tubule. The latter joins with others to form a larger one which receives further branches. × 15.

Fig. 2. First convoluted tubule (case 1 C) filled with haemorrhage. The tuft is detached. × 15.