METHODS FOR THE COLLECTION OF FLUID FROM SINGLE GLOMERULI AND TUBULES OF THE MAMMALIAN KIDNEY

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Accepted for publication July 20, 1941

In 1921 Wearn and Richards (1) demonstrated the possibility of collecting fluid from single glomerular capsules in the living frog's kidney. In the 20 years which have succeeded this demonstration, methods have been gradually developed for the collection of fluid from single tubules of the amphibian kidney and for the quantitative analysis of the minute amounts of fluid obtained. The body of evidence accumulated by the application of these techniques has lent clear and decisive support to the filtration-reabsorption theory of urine formation (2). Glomerular fluid has been shown to have the composition of an ultrafiltrate of blood plasma in the eleven respects in which it has been examined. Reducing substances, chlorides and fluid have been shown to be reabsorbed from this filtrate as it passes through the tubules, and the locus of these reabsorptive processes has been established. The extension of this type of experimentation from the amphibian to the mammalian kidney has seemed desirable, not alone because the truth of the filtration-reabsorption theory could again be subjected to thorough examination, but also because the functional and anatomical differences between kidneys of the two types made it certain that new information would be disclosed. Progress has been made in this extension and the present paper describes methods which have proven successful in collecting fluid from single glomeruli and tubules of the mammalian kidney.

Preparation for visualization of the kidney surface. A majority of the experiments have been performed on guinea pigs and rats. In both species, a unilateral (right) nephrectomy was done from 5 to 43 days before the experiment in order to produce enlargement of the nephrons in the remaining kidney. This operation was performed on 89 guinea pigs and 56

1 The expenses of this work have been defrayed in large part from a grant by the Commonwealth Fund of New York. A preliminary report of the investigation was made to the American Physiological Society in April, 1941 (This Journal 133: 480, 1941).
rings under aseptic precautions and with ether anesthesia. If it is assumed that the weight of the two kidneys was originally the same, hypertrophy of the remaining kidney was apparent by the fifth day post-operative, became maximum during the third week when it averaged 71 per cent in the rats and 62 per cent in the pigs, and remained stationary after that time. The subsequent procedures differed somewhat in the two species and must be described separately.

*Guinea pigs.* The guinea pigs, adult females weighing between 400 and 600 grams, were injected intraperitoneally with 0.45 cc. (29 mgm.) of sodium pentobarbital. Ether was given when necessary during the preparation. Sodium barbital, chloralosane and urethane were substituted for pentobarbital in a few experiments. The animal holder was a copper plate embedded in a sheet of cork and heated from beneath by a 15-watt bulb; the animal’s body lay on the plate, and the cork provided attachment for the retractors and for the pins which restrained limbs and head. The trachea was isolated for the subsequent insertion of an 18-gauge needle, permitting insufflation with oxygen, and the right jugular vein was cannulated and connected with a burette containing a solution of 0.9 per cent sodium chloride or 10 per cent sucrose. Blood pressure was not usually measured. After the abdomen was opened by a mid-line incision, the entire gastro-intestinal tract was removed following successive ligation of the coeliaco-mesenteric axis, inferior mesenteric artery, esophagus, and gastro-hepatic omentum. The urinary bladder was emptied. Fat and peritoneum were removed from the lower pole of the kidney surface. The renal capsule was usually left in situ since it did not interfere with visibility or puncture of the surface units and since, if it were removed, the visible blood vessels became dilated, the surface covered with a layer of protein-rich fluid, and puncture was more apt to result in tears of the tubule walls or hemorrhage from the vessels which border them. The abdominal wall was retracted and raised, and the abdominal cavity filled with liquid petrolatum (light), warmed to body temperature, which covered the kidney to a depth of about 5 mm. This oil prevented the kidney from drying, aided in the diffusion of light, and could be readily distinguished from the watery fluid that originated in the nephrons. Its temperature remained between 35° and 39°C. during the experiment.

The surface of the kidney was illuminated by a 300-watt bulb focused on the butt end of a lucite rod 12 inches in length, 0.5 inch in diameter and ground down at one end to a bevelled tip 0.08 inch in diameter. When this tip was brought into contact with the oil by a micromanipulator, a considerable portion of the kidney surface was brilliantly illuminated but pulsatile and respiratory excursions made it impossible to visualize details of structure. Movements of the former type were diminished by exerting downward and lateral pressure on the upper pole of the kidney with a glass
rod, terminating in a disc one-half inch in diameter and shaped to fit the kidney surface; if the movements were still too gross, a small area could be immobilized by exerting downward pressure with the lucite rod at a point close to the glass disc. The movements due to respiration were particularly troublesome in the guinea pig and after attempts to inactivate the diaphragm and to fix or support the kidney proved unsuccessful, recourse was had to paralyzing the respiratory muscles with curare. One milligram of curare,\(^2\) injected subcutaneously, abolished movements within ten minutes and oxygenation was maintained by intra-tracheal insufflation with 100 per cent oxygen. Under these circumstances the kidney was completely immobile and, examined with a binocular microscope at 85 magnifications, presented the appearance illustrated by the drawing of figure 1.

During the first hour after the preparation was completed, the kidney

\(^4\) The specimen employed was supplied by the courtesy of Dr. Hans Molitor of the Merck Institute for Therapeutic Research.

Fig. 1. Appearance of the ventral surface of a guinea pig's kidney when observed by the methods described in the text. India ink has been injected into a single tubule segment and has filled the 3 coils of a proximal convolution shown at 7 o'clock. The rounded interruption of the tubule pattern at 12 o'clock is a glomerulus. Photograph of a drawing made from life by Miss Edna Hill through the courtesy of the Harrison Department of Surgical Research, University of Pennsylvania Medical School. Magnification, approximately 50X.
continued to form urine at the rate of about 1 cc. per hour and retained its ability to reabsorb glucose and fluid, for the urine was practically free of fermentable reducing substances and contained exogenous creatinine in concentrations averaging 47 times that in blood plasma. Urine and blood specimens were collected at the beginning and end of each experiment by direct puncture of the bladder and vena cava with glass capillary pipettes containing, in the latter instance, an anticoagulant.

The chief disadvantage of the guinea pig preparation was the brevity of the period during which normal kidney function persisted. Within 1.5 hours after respiration was arrested the surface tubules were no longer distended with fluid, their epithelial lining became white and more readily visible, and the surface blood vessels showed brief cycles of contraction and relaxation (about 5 and 15 sec. respectively) which were apparently due to concurrent changes in some major renal vessel since they were accompanied by gross alterations in kidney size. When the blood pressure was measured at this time it was usually found to have fallen markedly. A second disadvantage was the considerable decrease in plasma concentrations of glucose and exogenous creatinine which occurred during the period of observation. This decrease, in the case of glucose, was especially marked after phlorhizin administration and might have been anticipated from the exclusion of the hepatic circulation but, in the case of creatinine, its mechanism was less clear.

Rats. The rats, adult males weighing between 300 and 400 grams, were starved for 18 hours preceding the experiment. They were anesthetized by the esophageal injection of 2.0 grams of urethane per kgm. in 10 cc. of tap water. The trachea was isolated, the jugular vein cannulated, and the abdomen incised in the mid-line as in the guinea pigs. The viscera were not removed but simply withdrawn from the vicinity of the kidney by retractors. The bladder was emptied, the abdominal wall supported, the abdominal cavity filled with oil and the kidney illuminated as in the guinea pigs. Blood specimens were taken by cutting the tip of the animal’s tail. It proved unnecessary to arrest respiratory movements; if they were troublesome, the intravenous injection of 100 mgm. of sodium barbital usually diminished them to a point where they, as well as the pulsatile movements, could be controlled with pressure by the glass disc and lucite rod. This preparation was superior to that of the guinea pig in many respects. The entire operation could be completed in 15 minutes, the kidney was not touched, the concentrations of glucose and exogenous creatinine in blood plasma remained relatively constant, blood could be obtained at any time during the experiment, and the animal’s condition continued good for 3 hours or more. A single advantage was retained by the guinea pig. In that species alone have we been able to see functioning glomeruli.
Collection of fluid from glomeruli. It seemed essential to the success of this investigation that we obtain at least a few specimens of glomerular fluid. Persistent attempts were therefore made to visualize glomeruli on the kidney surface of a variety of animals. The earlier of these attempts\(^3\) amply confirmed Bowman's statement, made in 1842, that "the Malpighian Bodies are rarely if ever visible quite on the surface of the kidney" (4). The topographical relationship of typical superficial nephrons to the kidney surface is shown in figures 6 to 10 (insets) where the glomeruli are seen to be covered by several layers of proximal convolutions. Even in the skunk, where glomeruli often lie within 0.2 mm. of the kidney surface, they are invisible by our methods of examination. In the guinea pig, however, we have had some measure of success.

In about one out of four guinea pigs, when the entire anterior surface of the kidney was examined, one or more round objects were seen which proved to be glomeruli. In the rare instances when these glomeruli lay

\(^3\) The kidney surface of the following animals has been examined during life in an attempt to visualize glomeruli: bat (2), adult cat (1), cat aged one month (1), ferret (1), adult white mouse (1), new-born white mice (4), deer-mouse (1), muskrat (1), adult opossums (25), pouch opossums from 2 to 7 weeks after entering the pouch (4), rabbits (8), adult rats (5), new-born rat (1), skunks (7), and gray squirrel (1). No glomeruli were observed in any of these animals except in one opossum kidney within an area of focal nephritis and in one skunk kidney which was examined postmortem. Large round objects were seen in the younger pouch opossums but histological examination did not suggest that these were functioning glomeruli. We varied our method of illumination by placing the lucite rod beneath the kidney, as is possible in the bat, by thrusting it into the kidney substance and into the cysts which occasionally occur in the opossum kidney; no one of these alterations made the glomeruli visible though, in many of the animals examined, some were demonstrated to lie within a millimeter of the kidney surface. Two further variations in technique were employed in skunks, opossums, and rabbits. In the first we attempted to color the glomeruli so that their visibility might be improved; the abdominal aorta below the renal arteries being ligated and the coeliac axis cannulated, a 0.1 per cent solution of Janus Green B was injected during momentary arrest of the renal circulation; although the glomeruli proved to be well colored when the kidneys were sectioned, they were not visible from the kidney surface. Finally it was determined to expose the sub-surface glomeruli by removing a slice of kidney tissue. Either at a preliminary operation or at the time of the experiment, a thin section was removed from the kidney surface by a razor, hemorrhage being arrested by momentary clamping of the renal artery and by the application of the cut edge of a piece of skeletal muscle. A large number of glomeruli became readily visible but we were unable to collect any fluid from them and they did not appear to be functionally active. The glomerular capillaries were dilated, the corpuscles within them stagnant, and this appearance did not change when the renal blood flow was interrupted or when adrenalin or sucrose was injected intravenously. Moreover, the cut surface of the kidney was covered with a layer of exudate or tissue fluid. The technique was discarded as being grossly unphysiological, a decision which may have been premature since Ellinger (3) has described the appearance of fluorescent dyes in sub-surface glomeruli of rats exposed by an identical procedure.
completely on the kidney surface, the individual capillaries could be identified and blood could be seen flowing through them. We have only seen six such glomeruli in nearly 100 animals. There was no visible clear capsular space about them, as is the case in amphibia, and when they were punctured by a quartz pipette a capillary was torn with consequent hemorrhage from the tuft followed by permanent stasis. On one occasion puncture was accomplished without hemorrhage but apparently resulted in contraction of the afferent vessel, for all of the capillaries emptied themselves of blood and remained contracted. No one of these glomeruli showed intermittence of blood flow, but only six were observed and no one of them for over 5 minutes. The majority of glomeruli seen were less clearly visualized but proved more suitable for our purpose. They appeared as rounded reddish objects, either below the kidney surface and covered by a single layer of tubules, or as a structureless interruption in the pattern of surface tubules (fig. 1). These glomeruli could usually be punctured without hemorrhage and, in 7 experiments, sufficient fluid has been collected from them for analysis. The puncture was performed with a quartz pipette, about 7μ in internal diameter at its tip, attached to a glass rod and micromanipulator in the fashion which has been described (1). The pipette was filled with mercury except at its extreme tip, where a small quantity of a light oil colored with Scharlach-R had been introduced. When the glomerular capsule had been penetrated, the oil was injected; at first it filled and distended the capsular space and then flowed on into the proximal tubule which might or might not reach the kidney surface. As soon as a millimeter or so of the proximal tubule had been filled with oil, the oil remaining in the capsular space was evacuated into the pipette and the collection of glomerular fluid commenced against a slight positive pressure in the collecting system. The oil served the double purpose of identifying the punctured object as a glomerulus and, by its continued presence in the proximal tubule, gave assurance that the collected fluid was derived from the glomerulus rather than from any more distal portion of the nephron. The collection was continued until sufficient fluid had been secured for analysis or until it was terminated by some accident. In the 7 completed experiments the average amount of fluid obtained was 0.24 c.mm. Subsequent dissection of the nephron, by the methods to be described, proved whether or not any tubule bordering the glomerulus had been accidentally punctured during the experiment.

Two matters should be mentioned since they may detract from the significance of these experiments. Glomerular fluid, collected from amphibia, proved to be free of protein (5). Of the 6 specimens of this series which

4 The method cannot consistently detect concentrations below 0.03 per cent. The statement, in this paragraph, that a specimen contained no protein should therefore be taken to mean that it contained less than 0.03 per cent.
were similarly tested only two were negative, two contained between 0.15 and 0.20 per cent, and two contained amounts estimated at 0.80 per cent. This need not mean that the normal mammalian glomerulus excretes any considerable amount of protein, for the great majority of tubule fluid specimens and two from within 1 mm. of the glomerulus were protein-free. But it does suggest that glomerular capillaries leak protein readily even in the absence of gross damage, and that the glomeruli in question were affected either by the act of puncture or by the injection of oil. In the second place, the rate at which fluid was collected was about 30 per cent lower than would be anticipated on the basis of calculations made from creatinine clearances (6), the average figure in the 7 experiments being 0.7 c.mm. per glomerulus per hour. While the collections may have been incomplete due to leakage of fluid through tears in the glomerular capsules, these slow rates may suggest that the surface glomeruli, after puncture at any rate, were less active than those which lay deeper in the kidney substance.

The collections of glomerular fluid therefore were neither very numerous nor wholly free from objection. Fortunately the conclusions to which they led received support from the more numerous and satisfactory experiments upon proximal tubules.

Collection of fluid from proximal convolutions. As will be understood from an examination of figures 5 to 10, the vast majority of the tubule segments appearing on the kidney surface proved to be portions of the proximal convolutions. In a good preparation they were distended with fluid and presented the appearance illustrated in figure 1. Each segment looked precisely like its neighbor and it was impossible to distinguish anatomical portions of the proximal tubule from each other or from the distal tubule by simple observation. Two juxtaposed segments were not necessarily portions of the same nephron and their relationship to each other could only be shown by such an intratubular injection as is illustrated in figure 1 where india ink was used. The tubules were larger in adults than in young and increased in size after unilateral nephrectomy. Of the animals examined, they were largest in the opossum and skunk. In the guinea pig the continuous segments on the surface were rather longer than in the rat. An active circulation could be seen in the blood capillaries which bordered each segment and, on occasion, crossed over a tubule. If the circulation failed, or if the renal artery was clamped, the lumina of the tubules collapsed and their walls became more apparent; under these circumstances some tubules appeared white while others showed brownish granulations but these differences did not prove to be characteristic of any particular portion of the nephron. If the tubules were collapsed when first observed, they could be promptly distended by the intravenous injection of 1.0 cc. of a 10 per cent sucrose solution. The effect was much more marked than that pro-
reduced by a similar amount of 0.9 per cent sodium chloride solution, and suggests that sucrose affects fluid reabsorption in the proximal tubule.

Any dilated surface tubule with its axis parallel to that of the pipette was selected and the point thrust into it; if the point were properly bevelled and the renal capsule not too thick the insertion could be made without tearing the tubule wall, damaging its capillaries, or penetrating into a deeper layer of tubules, though these accidents often occurred. The most perfect punctures were made in the rat by simply pressing the pipette tip against the tubule wall and allowing the small respiratory excursions to tease a hole for it. With the pipette inside the tubule lumen a short column of red oil was injected; this demonstrated the proper position of the pipette and, if it subsequently moved along the tubule into further convolutions, proved that there was a flow of fluid down the tubule. When the oil column had taken up a position distal to the site of puncture, the collection of fluid was commenced, the pressure in the collecting system being so adjusted as to immobilize the oil. The immobility of the oil column provided assurance that all of the fluid descending the tubule was being collected and that there was no contamination by fluid which had passed distal to it. In a few experiments metallic mercury has been substituted for the oil; this provides a more certain block but has been impossible to inject with consistent success.

There can be no doubt that fluid entering the pipette originated from within a nephron, for no appreciable amount of fluid can be collected from the normal kidney surface or from the kidney substance unless the pipette be thrust into a tubule lumen or damage a blood vessel. We have seen no indication of large currents of interstitial fluid (7) and there are no obvious spaces on the kidney surface unoccupied by tubules or blood vessels.

A number of factors combined to make the collections less simple than this description perhaps suggests. In the first place, the mere fact that the tubule appeared distended with fluid did not necessarily mean that there was an active flow within it, for frequently the injected oil would move onwards sluggishly or not at all; under these circumstances other tubules were punctured until one was found from which fluid could be collected. A fluid collection, once begun, continued steadily unless some extraneous factor intervened. In this observation and in the observation that pulsatile movements at the proximal end of the oil column, apparently transmitted to it from the glomerulus, continued without interruption, we find arguments against the existence of intermittent glomerular activity in our preparations. A second difficulty lay in the very high intratubular pressure which was particularly prominent during the infusion of hypertonic sucrose solutions. Under these circumstances the injected oil column, which at first had started down the tubule, would rush suddenly and violently back towards the pipette and emerge on the kidney surface. We
attributed this to a leak at the point of puncture and the consequent collapse of the punctured nephron by its distended neighbors. When saline infusions were substituted for sucrose, or all infusions omitted, the difficulty was encountered less often. A third difficulty lay in the tendency of the pipette point to become obstructed by some particle within the lumen or by contact with the tubule wall. When this occurred, the oncoming fluid either pushed the oil column down the tubule or emerged on the kidney surface; in the latter event it often came with sufficient force to detach the capsule and form a visible drop of fluid around the point of puncture. The collection could be continued if the pipette were withdrawn from the lumen and the fluid picked up as it emerged from the tubule and lay beneath the oil which covered the kidney surface. Fourteen per cent of our collections have been made in this fashion. We deprecate the technique, for there might have been an admixture with the thin layer of surface fluid which may cover the kidney, but two points minimize the dangers introduced by this fluid. It was only present in considerable amounts when the renal capsule had been removed and in the immediate vicinity of the points upon which pressure was being exerted. It was readily distinguished from tubule fluid since it contained about 1.0 per cent protein, and reducing substances and chloride in the concentrations anticipated in an ultrafiltrate of blood plasma.

We have described the technique employed in the collection of fluid from any dilated surface tubule, chosen at random. Almost invariably these tubules proved to be proximal convolutions and in the middle third of this segment. The last third dips deeply into the kidney substance to join the loop of Henle, and is inaccessible by our technique (figs. 5–10). The first third appears occasionally on the surface and a special procedure was devised to identify it. A pipette containing air below the mercury and, at its extreme tip, a little oil, was thrust into a surface tubule. After the oil was injected and allowed to flow distal to the punctured point, a column of air was forced into the tubule. The air, prevented from going distally by the oil column, distended and outlined the coils of tubules on the glomerular side of the puncture. When the proximal end of this air column happened to be in a surface tubule this point was selected as the site of collection, with the assurance that it was on the proximal side of the original segment by the length of the air column. The technique was demanding but it provided a number of collections from the first third of the proximal tubule. It had the disadvantage that the sudden distension with air appeared to damage the tubule wall; during the subsequent experiment this portion of the tubule often looked white and some cellular detritus was found in the distal tubule at the time of its dissection. We have not felt that this damage disqualified the experiment, for the collection was made proximal to the region which had been distended. No evidence of similar damage from the
simple act of puncture or the injection of oil has been seen, other than the local tear in the tubule wall at the point of penetration. The glomerulus and the portion of the tubule proximal to the site of collection remained completely untouched and usually well below the kidney surface.

These methods for the collection of fluid from proximal tubules have proved quite satisfactory, especially in the rat. The distal tubules have presented particular difficulties, as yet only partially solved.

Collection of fluid from distal convolutions. Scattered over the surface of both guinea pig and rat kidneys there are occasional distal tubules which, coming to the surface, make but a single loop there and then descend again into the kidney substance (figs. 5-10). Their infrequency in comparison with proximal convolutions is indicated by the fact that only 3 out of 92 tubules, punctured at random, proved to be distal segments. Some special method of identification had to be designed for, though their diameter is smaller than that of proximal convolutions in a ratio of 2:3 when the measurements are made after death in dissected specimens (table 1), this difference is either absent or indistinguishable during life when the tubule lumina are dilated. We attempted to utilize the preferential vital staining of proximal convolutions by trypan blue in several series of experiments on guinea pigs and rats, hoping that the distal segments would appear as unstained tubules against a blue background. The attempts were unsuccessful for, though differentially stained, the contrast between the two segments could not be seen in the living animal.

It has, however, proved possible to identify distal tubules by the use of phenolsulfonephthalein. Six milligrams were injected intravenously and, 5 minutes later, the ureter was clamped to produce a maximum concentration of the dye. Under these circumstances the lumina of scattered tubules throughout the field became deeply colored and this color was particularly well seen when the tubule, ascending directly to the surface, allowed one to observe a column of fluid in depth. These colored tubules have uniformly proven to belong to distal convolutions. One such tubule was selected, its position relative to its surroundings noted, the clamp removed from the ureter, and puncture subsequently performed. Identification by this method involving, as it did, coloration of the tubule fluid made certain analyses impossible. The collection of satisfactory specimens has also been complicated by the small amounts of fluid available and the difficulty of establishing an adequate block. In 3 successful experiments the average volume collected has been only 0.07 c.mm. and its rate of collection 0.31 c.mm. per hour. While such small volumes were anticipated on theoretical grounds and were consistent with the demonstration of fluid reabsorption in the proximal tubule (6) they have imposed added difficulty on our analytical technique. When an oil column was injected into a distal tubule it would move onward in the direction of a collecting duct but then,
as collection of fluid was started, would return to the pipette point and often take up a position proximal to it. Under these circumstances of course the collected fluid was derived from distal to the point of puncture and the results of its analysis became meaningless. This difficulty was presumably associated with the low intratubular pressure and the extremely slow rate of flow which exists within the distal convolutions. Our methods for identification of distal tubules and for the collection of fluid from them are, then, still imperfect.

Methods of identifying the site of puncture. The collection and analysis of fluid possessed little or no significance unless the site of the collection could be accurately determined. The problem presented difficulties. In the earlier stages of the investigation attempts were made by our former colleague, Prof. Rudolf Kempton of Vassar College, to identify the punctured tubule by examining serial sections of a block of kidney tissue which contained the nephron in question. The method was very time-consuming and rarely provided more information than that the puncture was in a proximal or distal segment. Recourse was therefore had to the technique of maceration and dissection.

At the conclusion of each experiment, a pipette containing a 1:10 dilution of "soluble" india ink was reinserted into the tubule from which fluid had been collected, and a small quantity of ink injected. The kidney was then placed in 10 per cent formaldehyde. After two or three days of fixation it was removed and the small ink spot lying within the tubule and beneath the capsule around the puncture wound was located with a hand lens. A wedge shaped segment of kidney extending well into the papilla and containing the punctured nephron was then excised, care being taken to leave about 5 mm. of tissue around the central-lying ink mark. This block was placed in a stender dish containing concentrated hydrochloric acid and allowed to macerate until sufficiently softened for dissection. The length of the time required depends on the room temperature and the specimen therefore must be carefully watched, for a few hours' excessive maceration will destroy the structure of the nephrons completely. Under the conditions of our laboratory from one to three days was found to be most favorable. The acid was then poured from the softened tissue and it was rinsed by decanting with several changes of distilled water.

Dissection and isolation of the nephron was done under water beneath the binocular microscope at a magnification from 20 to 60 times, in the same dish which had contained the specimen from the beginning of the maceration. Strong direct light against a black background was found most useful during dissection, though transmitted light proved helpful in locating the ink within the tubule. Steel needles, frequently cleaned with an emery cushion to avoid the stickiness of the softened tissue, were used to untangle the marked nephron. No description of this procedure is possible, but its
progress is shown in figure 2. The most hazardous part of these manipulations was the final separation and disentanglement, without breakage, of the distal convolution from the redundant coils of the surrounding proximal convolution (fig. 2d).
Several features of the present experimental problem impose especial difficulties in the dissection of the punctured nephrons. The first and greatest of these is the disturbing fact that it is one specific nephron that must be successfully manipulated and not, as in most morphological work, a representative specimen of no peculiar value. Secondly, since quantitative measurements are to be made on the final specimen, the nephron must be isolated completely and in continuity from the glomerulus to the collecting tubule. The tenuous thin portion of Henle's loop may break at one point but no portion of it can be lost. Thirdly, the tortuous loops of

Fig. 3. The terminal portion of a proximal convolution from the kidney of a guinea pig (expt. 55 (6)). The arrow points to the small nick where the pipette entered. From this point downwards the lumen of the tubule can be distinguished and in it are oil droplets of varying size. The apparent break in the tubule is caused by an opaque rod which was laid on it for purposes of immobilization. Magnification 32X.

the convoluted tubules must be straightened out sufficiently, yet not broken, to allow an inspection of their surface throughout their entire length so that the point of entrance of the pipette with its ink mark can be examined, and the remainder of the tubule inspected for possible evidence of accidental trauma to some point other than the original insertion of the pipette. The position of the oil droplets blocking the lumen must also be determined and the adequacy of the blocking estimated by their size and number. Finally, all these manipulations were made more difficult in the experiments under consideration by the presence in the lumen of the tubule of oil or mercury. In the former case the tubules, normally of the same
specific gravity as the suspending water and therefore motionless, strove constantly to rise to the surface entangling themselves in the dissecting needles; in the latter they sank inert and heavy to the bottom of the dish and broke into fragments if roughly disturbed from this anchorage.

Fig. 4. The complete nephron from experiment 16 (6). Oil droplets are seen distending the tubule lumen throughout the lower four-fifths of the proximal convolution. There are no droplets in the remainder of the nephron. This relatively small nephron came from an unhypertrophied normal guinea pig kidney. Magnification 32X.

When these difficulties had been surmounted stereoscopic photographs at a magnification of 16 and 23 times were made. This gave an objective and permanent record of the morphological findings, as it is impossible to mount and preserve the delicately fragile original specimen. In most cases the photographs showed clearly the hole in the tubule at the point of entry of the pipette (fig. 3) and the occluding droplets of oil (fig. 4).

Limitations of depth of focus are noticeable however when photographing
a floating specimen, so that photographs are unsuitable for quantitative measurement. For this purpose a camera lucida outline drawing of the nephron at a magnification of 80 times was made, showing the point of pipette entrance and the site of oil and ink (figs. 5–10). In its preparation

Figs. 5, 6, 7. Camera lucida drawings of representative nephrons after microdissection showing the point of entrance of the pipette (arrow) and oil droplets in the tubule lumen. The small inserts show the position occupied by the loops of the convoluted tubules before microdissection. Figure 5, guinea pig; figure 6, guinea pig, experiment 13 (6); figure 7, rat, experiment 9 (6). Magnification 23X.
Figs. 8, 9, 10. Camera lucida drawings of representative nephrons after microdissection showing the point of entrance of the pipette (arrow) and oil droplets in the tubule lumen. The small inserts show the position occupied by the loops of the convoluted tubules before microdissection. Figure 8, rat; figure 9, guinea pig, experiment 55 (6) (cf. fig. 3); figure 10, rat, experiment 58 (6). Magnification 23X.
a source of error in measurement, namely, the effect of foreshortening produced by the coiling of the loops of the convoluted tubules, was removed by placing thin glass capillary tubes across the floating tubule so that its loops were flattened down against the bottom of the dish in a single plane.

The actual measurement of the length of the various portions of the nephron was made on this drawing by means of a map measure with a small wheel with which the straightened and flattened twistings of the tubule could be easily followed. Measurements of the diameter of the tubule in various segments were made on the actual specimen at a magnification of 43 times with a filar micrometer. The mean of at least ten measurements was used as the final expression of the diameter, a value sufficiently accurate for the purpose of these experiments, though it must be recognized that such an evaluation ignores the fact that in the hypertrophied proximal convolution the straight terminal portion, comprising about one-third of its length, may be twenty-five per cent thicker than the mean diameter of the convolution as a whole. A summary of these measurements is shown in table 1.

Besides the measurement of the length of the various portions of the nephron, the distance of the point of entrance of the pipette from a glomerulus was also recorded by means of the map measure.

This completed the morphological data on the nephron. The final procedure, accomplished in part during the course of the earlier dissection, was the isolation and inspection of neighboring nephrons to make certain that they had not been entered by the pipette or torn so that a source of contaminating tubule fluid had been produced. Ink granules which had leaked from the punctured tubule lumen were found not uncommonly in the interstitial tissue and on neighboring tubules, but its presence on the surface of an intact tubule rather than in its wall or its lumen allowed an easy decision as to whether a possible contamination could have occurred. In five instances such accidents were found to have happened; tears were
seen in contiguous tubules or their lumens contained either ink or, more frequently, oil droplets which showed plainly that more than one nephron had been involved in the experimental puncture.

To sum up the advantages of the morphological procedure it may therefore be stated that an objective and permanent record which allowed accurate quantitative measurement was obtained; that the position of the point of entrance of the pipette into the tubule lumen relative to the remainder of the nephron could be accurately determined; that the adequacy of the oil block could be demonstrated and that errors due to entrance into more than one nephron, errors not appreciated during the course of the experiment, could be positively eliminated.

SUMMARY

Methods have been developed for observing the kidney surface in anesthetized mammals and for collecting fluid from single glomeruli, proximal tubules, and distal tubules. Sufficient amounts of fluid can be collected for quantitative analysis by ultramicro methods and the precise locus of the collection can be determined.

It is unnecessary, but a pleasure to us, to remark that these experiments are a direct and logical continuation of those initiated by Prof. A. N. Richards many years ago and to record his continuous encouragement and advice in their execution.

REFERENCES

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