# Studies on the Mammalian Kidney.

By

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With 3 plates and 29 figures in the text.

## Contents.

## Preface.

In publishing this investigation it is my pleasant duty sincerely to thank my teacher, the Rector of the University, Professor NILS VON HOFSTEN for the interest and helpfulness he has shown in every respect.

My thanks are also due to my teacher Professor SVEN EKMAN, and to the Director of the Zoological Institute, Professor SVEN HÖRSTADIUS.

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On some points concerning the statistical methods I have had the opportunity of consulting Professor GUNNAR DAHLBERG, for which I tender my thanks.

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*Ivar Sperber.*

# I. Introduction.

## General account of the form of the kidney and the nephrons.

In this paper descriptions are given of the macroscopical and microscopical anatomy of the kidneys of a number of mammals, and some pertaining problems are discussed. To make clear the terms used a general account of the structure of the mammalian kidney is given below as regards the features investigated.

**The form of the kidney.** The mammalian kidney consists of cortex and medulla. This division refers mainly to differences visible to the naked eye. The medullary rays are as usual included in the cortex, though their structure agrees in essentials with that of the medulla. The medulla usually consists of two layers, the inner zone and the outer zone (fig. 1 *A*). The outer zone may be subdivided into two parts, the inner stripe and the outer stripe (PETER 1909). (HEIDENHAIN 1937 includes the outer stripe in the cortex. He also creates an entirely new terminology for the other layers. This does not seem appropriate, as the inner boundary of the cortex in this case would be mainly indistinguishable macroscopically. As PETER's terminology has already been used by several writers and is suited to both macrosopic and microscopic investigations, alterations seem unnecessary.)

There is considerable variation in the shape and structure of the mammalian kidney. To facilitate the description of the forms, these may be divided into some types, descriptions of which are given below. These types mainly agree with those of previous authors, especially GERHARDT (1911, 1914).

1. The kidney surface is even, or has slight grooves. There is a medial concavity, the hilus, where the ureter and the blood-vessels enter the kidney. The hilus leads into a space, the sinus renis, which contains the renal pelvis (fig. 1 *A*, *pv*). The medulla is undivided and forms a papilla. The collecting ducts of the kidney open on the apex of the papilla, forming the area cribrosa (fig. 1 *A*, *a.c*). The papilla projects into the pelvis, which often has processes projecting into the medulla. Alternating with the processes there are elevations, somewhat like buttresses, at the basis of the papilla. These are often called pyramids, but to

avoid confusion, they are properly named secondary pyramids. (Other names are: Anbaue, Seitenwülste (GERHARDT), Nebenwülste (MÜLLER 1883), Nebenwarzen (HYRTL), Pseudopapillen (BRASCH 1909).) It may be mentioned that GERHARDT calls a pelvis with processes 'branched'; this can cause confusion.

2. Closely allied to the kidney with a papilla is a type where the

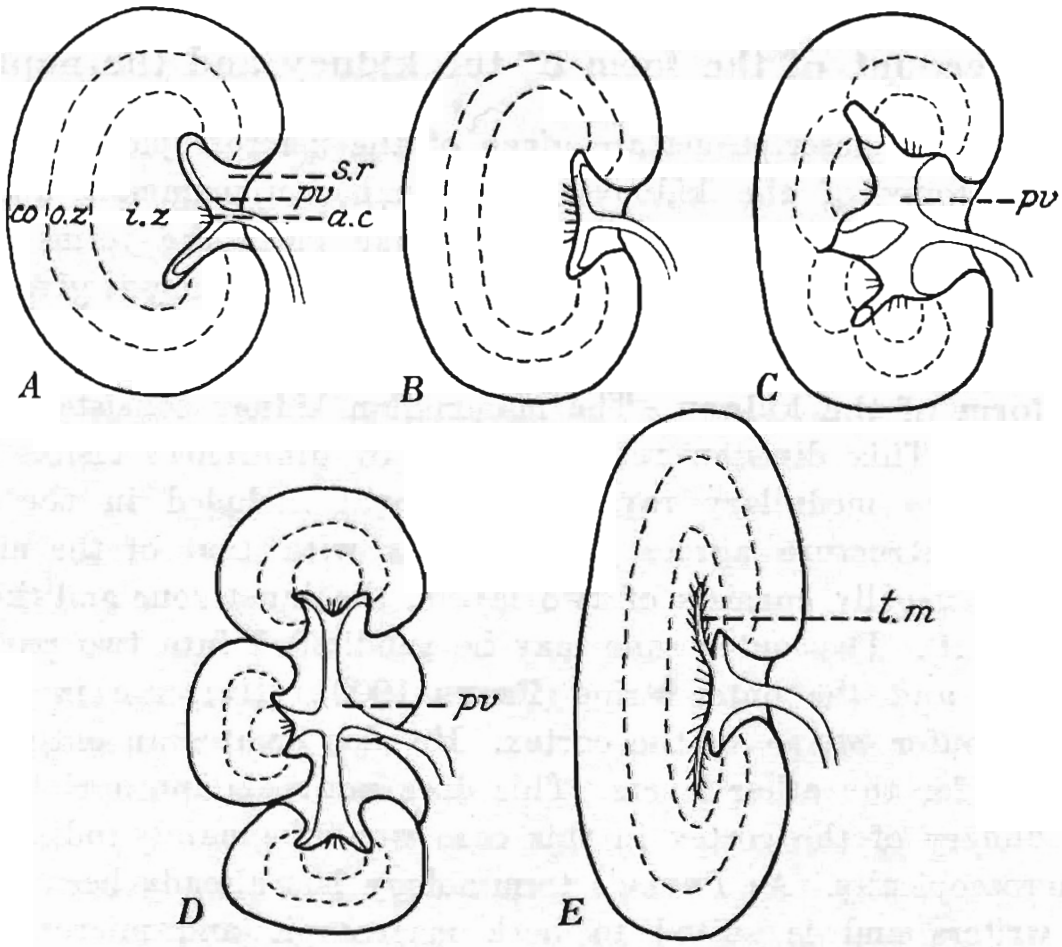


Fig. 1. The mammalian kidney types. *A* Simple kidney with a papilla. *B* Crest kidney. *C* Kidney with several papillae. *D* Renculi kidney. *E* Kidney with tubi maximi. *a.c* area cribrosa, *co* cortex, *i.z* inner zone, *o.z* outer zone, *pv* pelvis, *s.r* sinus renis, *t.m* tubus maximus.

papilla is replaced by a crest (fig. 1 *B*). The crest, too, may be absent, and then the area cribrosa lies on an even or concave surface, constituting the lateral limit of the pelvis. The area cribrosa is not round as in the preceding type, but oval.

3. The medulla is divided into two or more (primary) pyramids (fig. 1 *C*). Each pyramid forms a separate papilla or crest. The cortex, however, is undivided as in the preceding types.

4. Structurally, the kidney of this type consists of several parts, renculi, which are organized entirely as small kidneys (fig. 1 *D*). The pelvis is branched, as is often the case in the type with several papillae also. Externally, kidneys of this type are often lobated.

5. The kidney of this type is usually a simple kidney with even surface and undivided medulla. Part, at any rate, of the collecting ducts join large ducts, tubi maximi, mostly two, which open into the pelvis, in most cases at the extreme ends of the area cribrosa (fig. 1 *E*).

**The tubules.** The shape and course of the renal tubules have been investigated repeatedly in the nineteenth century. Later, especially PETER (1907, 1909, 1927) and HUBER (1911, 1917, 1928, 1935) have investigated this subject. PETER (1909) gives a full account of the earlier papers.

Below I shall give a general account of the tubules, mainly following PETER and HUBER. The terminology is somewhat altered, chiefly in agreement with GRAFFLIN (1937).

POLICARD (1912) is of the opinion that the subdivision of the nephrons made by PETER overreaches the observable facts. In the discussion on this matter I agree entirely with PETER. The differences between the segments which appear in maceration preparations cannot be explained as artefacts. They are far too regular. There is little difficulty in finding the distinct segments in sections, too (cf. also OKKELS 1929).

v. MÖLLENDORFF (1930) adopts POLICARD's division and states that this is better suited as a basis for comparative studies. This is possible but PETER's scheme comprises all different segments that are discernible, which is not the case with that of POLICARD. Thus PETER's system seems best suited to investigations within the *Mammalia*.

The tubules may be divided into the nephrons and the collecting tubules.

A nephron begins with Bowman's capsule, which surrounds the glomerulus. The form of the capsule is variable. If, as seen under the microscope, the diameter passing through the beginning of the proximal tubule is the longest diameter, the capsule is said to be oval. If this diameter is the shortest one the capsule is termed transversely oval, and the capsule is called round when the contour is more circular. The capsule may, of course, also be flattened, but as this is difficult to see, it is mentioned only when this circumstance is marked. The epithelium of the capsule is low and transparent.

The next part of the nephron is the proximal tubule (fig. 2 *A*). At the beginning of the proximal tubule there is sometimes a short constriction, the neck. The cells of the proximal tubule contain rod-like mitochondria, and possess a brush border. In maceration preparations this segment is distinguished by its high, relatively opaque epithelium. The first part of the proximal tubule mostly consists of some loops near the capsule, and is termed pars convoluta. The rest of the proximal tubule, the pars recta, descends in a more or less straight path towards the medulla.

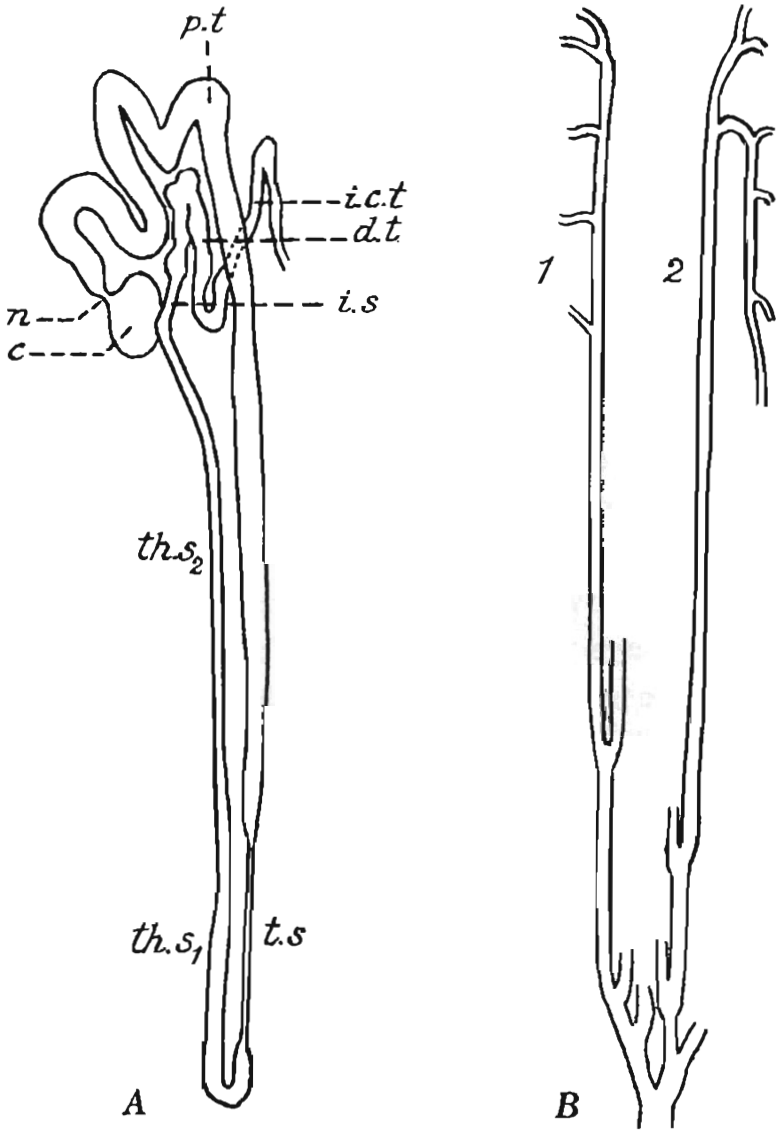


Fig. 2. Scheme of *A* nephron, *B* collecting tubules. *c* capsule of Bowman, *d.t* distal tubule, *i.c.t* initial collecting tubule, *i.s* intercalated segment, *n* neck, *p.t* proximal tubule, $th.s_1$ thicker part, $th.s_2$ thinner part of thick segment, *t.s* thin segment. *1* collecting tubule with direct junctions, *2* d:o with arcade.

The transition into the next segment of the tubule, the thin segment, normally occurs at the boundary between the outer and the inner stripe. The thin segment has a small diameter, and is very transparent, as its epithelium is very low and the granules are few.

The next part, the thick segment, again, presents a high epithelium, containing rod-shaped mitochondria, but without a brush border. This segment is often differentiated into a thicker proximal part and a thinner, more transparent, distal part. The thicker part is mostly situated in the outer zone, and the thinner part in the cortex. The transition is gradual.

Together with the pars recta of the proximal tubule the thin segment and the thick segment form the loop of Henle. The loops are said to be long when they turn in the inner zone of the medulla. Those loops which turn in the outer zone are called short, and if there are nephrons whose loops turn in the cortex, they are called cortical nephrons. In

the latter the thin segment may be absent. The long loops always turn within the thin segment, and the short loops mostly turn in the thick segment.

The thick segment returns to the capsule, and touching the capsule it passes into the distal tubule. This segment is tortuous and usually thicker and less transparent than the thick segment, but otherwise of about the same structure. The first part of the distal tubule, however, may show about the same appearance as the thinner part of the thick segment, and is then called the intercalated segment.

The transition into the next segment, the initial collecting tubule, is gradual and often indistinguishable. The epithelium of this segment is relatively low and transparent.

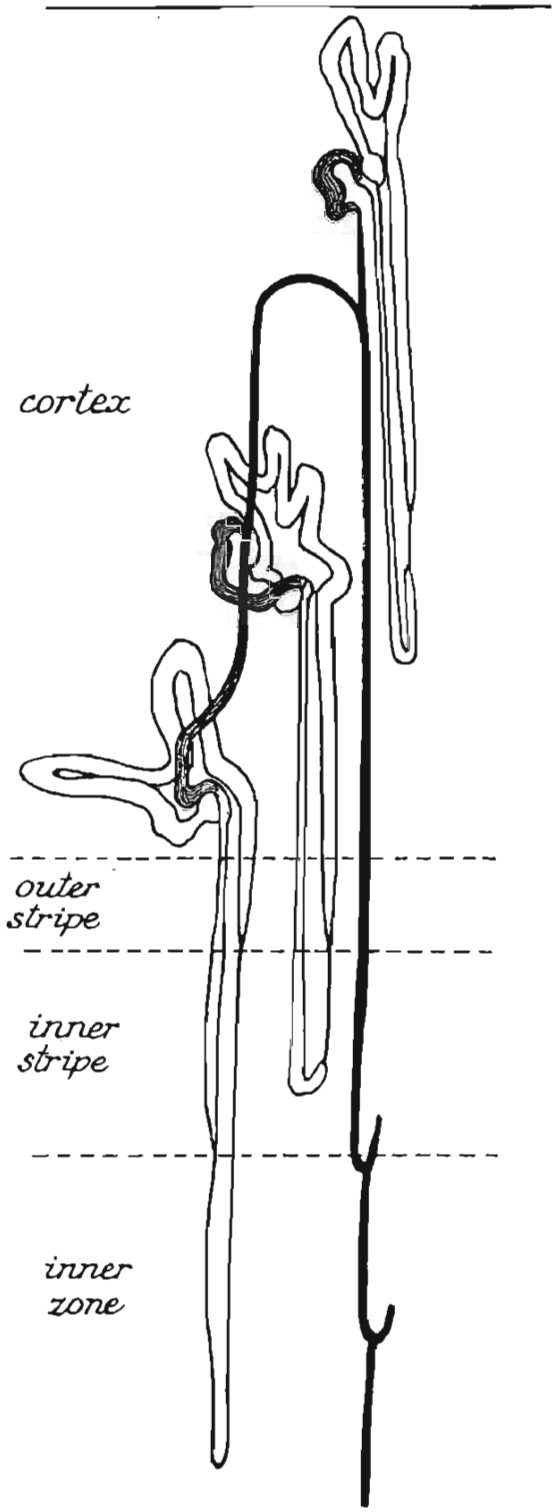


Fig. 3. Scheme of the situation of the nephrons. Collecting tubules black, distal tubules grey.

The initial collecting tubules join to form the collecting tubules, the epithelium of which, in the cortex, is quite like that of the initial collecting tubules. In the medulla it becomes higher and often somewhat opaque. In the inner zone the collecting tubules join repeatedly, mostly in pairs (fig. 2 *B*). The ducts thus formed open on the area cribrosa. The junctions between the initial collecting tubules and the collecting tubules are called peripheral junctions and occur in the cortex. In the medulla only collecting tubules join, and these junctions are termed central junctions. The peripheral junctions show two distinct forms. They may be direct, in which case the collecting tubule begins in the outer part of the cortex and descends to the area cribrosa, nephrons joining it in the cortex (fig. 2 *B*, *1*). In other cases the collecting tubule forms an arcade, that is, it starts in the lower parts of the cortex, ascends through the cortex, where some nephrons join it, and turns in the higher parts of the cortex to descend to the area cribrosa (fig. 2 *B*, *2*).

**The position of the nephron segments in the kidney.** The capsule, the main part of the proximal tubule, the thin part of the thick segment, the distal tubule and the initial collecting tubule are always situated in the cortex (fig. 3). In the outer zone are found the most central parts of the proximal tubules, thin segments and the thicker parts of the thick segments. Apart from collecting tubules, which are found in all layers, the inner zone contains only thin segments.

Normally, most proximal tubules end at the same level — the boundary between the outer and the inner stripe. Thus, the latter never contains any part of the proximal tubules. The zone boundary is the level at which the most centrally-reaching thick segments pass into thin segments.

As a rule the loop of a high (peripheral) nephron turns at a higher level than the loop of a deeper (more central) nephron. Small deviations from this rule are not unusual. The loops of the deepest nephrons get nearly as far as the area cribrosa.

## Technique and methods.

The shape and size of the kidneys has been ascertained from fresh kidneys, when possible. In most cases only preserved material could be obtained, and then both shape and size may be altered to a certain extent. Deformation is often evident as a result of the pressure from other organs during hardening. This affects the relations between the dimensions as well as the shape itself. The measurements may be altered by swelling or, mostly, by shrinkage. These changes, however, seem seldom to be so great that a good idea of the shape and size cannot be obtained.

The thickness of the layers and the form of the papilla has been assessed from a medial section, parallel to the flattened surfaces of the kidney. Such a section is in normal circumstances easily obtained by cutting through the cortex along the largest circumference of the kidney through the hilus, and tearing the halves of the kidney apart. Such a section then generally passes through the area cribrosa. The measurements of the medulla are made from the area cribrosa, transverse to the longest axis of the kidney. Such sections of a number of kidneys have been drawn as accurately as possible and are reproduced in the special section. Of course they are not always exactly median, as the kidneys are often somewhat irregular in shape. The boundaries between the cortex and the medulla and between the zones of the medulla have also been drawn in these figures, but sometimes these boundaries are slightly generalised as it is not always possible to trace them all over the section. In cases when it has not been possible to ascertain their essential features they have, of course, not been drawn. The shape of the pelvis has most often been examined by dissection.

To investigate the tubules, pieces of kidneys have been macerated with hydrochloric acid. This technique has been employed on both fresh and preserved material. Pieces of the kidney are placed in the concentrated acid (sp. gr. 1.19) and remain there for some hours, in the case of fresh material. The pieces are then transferred to a Petri dish, with water. After some time they are ready for teasing. The tubules are now normally fairly soft. If the acid is less concentrated the pieces must remain in it longer and the tubules become more brittle. It is usually advantageous to employ concentrated and slightly diluted acid on different pieces. Sometimes still better results may be obtained by injection of the acid through the renal artery (according to HUBER 1911). This often gives a very even and good result. The capsules, however, easily become detached later during dissection.

The acidity of the water in which the teasing is carried out is of some importance. If there is too much acid the tubules become brittle, and if there is too little the same result may ensue. It is, however, not possible to define the suitable acidity, as it is influenced by several factors, as age of the animal and the maceration technique.

Sometimes the preparation is more suited for teasing some days later, and often the condition of the tubules may be improved by adding a few drops of glycerine to the water, especially when the tubules are brittle. Preserved kidneys sometimes give very good macerations, but mostly the results are inferior to those obtained from fresh material. This seems especially to be the case when the kidneys have been exposed to light for considerable periods.

The tubules are dissected with glass needles. If the shape only is to be examined, it is preferable to macerate with slightly diluted acid. Material macerated with concentrated acid lends itself better to measurements, however. Long segments are then easily dissected, and often it is possible to isolate complete nephrons. The coils and loops of the tubules are also more easily stretched out, which is necessary for tolerably exact measurements.

The first measurements are made already during the dissection (under a dissecting microscope). As soon as the thin segment of the nephron under dissection is clearly recognised, it is measured with an eyepiece micrometer. When the nephron or segment is isolated it is transferred to a slide with the aid of a wide pipette. The diameters of the capsule are measured with the nephron still floating in water on the slide. Then most of the water is removed so as to stretch the tubule out on the slide. The lengths of the segments of the nephrons are now measured with the eyepiece micrometer at a magnification of about 200 ×. The thickness of a segment is obtained as the mean of several measure-

ments at separate points, at a magnification of about 350 ×. Sometimes the nephron or the segment has been drawn at a known magnification, and the measurements have been made from the drawing.

The following sources of error seem to be most important:

1. The tubules shrink or swell during maceration or later. This is almost always the case. After maceration with weak acid there is generally some shrinkage, and it may be great in some cases. Swelling most often occurs after maceration with strong acid. The acidity of the water in which the dissection takes place has, however, the most striking effect. A segment may exhibit variations to an extent of 50 per cent due to this factor. The extreme deviations are of no importance as a source of error, however, since in these cases the material is not fit for dissection.

To eliminate this source of error as far as possible the following procedure has been used: On the piece that is to be macerated the thicknesses of the cortex and medulla are measured, or their sum. Immediately before teasing the same measurements are made. Thus, gross variations in the thickness of the layers are discovered and measured. These measurements are, however, not very exact, and there is probably not a strict relation between the changes of the different segments as their structure is different. Considering this, deviations less than ten per cent of the initial value have been disregarded. Greater changes are mentioned in the description of the tubules in the special section, and in calculations of absolute values a correction has been applied.

Earlier investigators have not tried to estimate this source of error. PETER and his co-workers (1909, 1927) and HUBER (1911, 1917) seem to have believed the dimensions of the tubules to be the same after maceration as before. FOOTE and GRAFFLIN (1938), on the other hand, are aware of the probability of changes, but make no attempt to obtain the true values.

Swelling and shrinkage may, of course, occur after dissection also. Especially during drawing, and if the tubules are subjected to long series of preparations, relatively large variations may occur which are not possible to assess. This is probably the case when the measuring is preceded by mounting in glycerine-jelly (STEINBACH 1926, PETERS 1928). These errors are probably best avoided by measuring the tubules lying in water on the slide direct with an eyepiece micrometer. It seems to me that this should more than compensate the somewhat lower degree of exactness in measuring of this method as compared with the measuring from drawings.

2. The length and thickness of the tubules may be altered by the teasing. The tubules, however, are normally so elastic that they resume

their earlier shape even after severe distortion. The thin segment seems to be most liable to permanent lengthening, and to control this, this segment is measured as soon as possible (cf. p. 259). When the connective tissue is tough and the tubules are very soft after maceration the other parts of the nephrons may also be influenced by this factor, and such material has been avoided, if possible.

The capsules are very easily flattened through any pressure. This I have tried to avoid by measuring the capsules floating in the water, but it is possible that the largest capsules have been somewhat flattened all the same. The latter also have a tendency to present their largest surface to the observer when they lie on a slide, which also tends to raise the values of their dimensions as compared with the true values.

3. The length of the loops is underestimated, as they do not all lie in the same plane. This applies mainly to the proximal and the distal tubule. This source of error is diminished by the use of relatively soft material. (The error due to foreshortening must have been considerable in the measurements of PETER, as he realises, and is probably considerable also in the data given by PAI 1935.)

4. The deficiency of exactness in the measuring. This factor is important, but duplicate measurements normally agree to within 5 %. This error is diminished by measuring a greater number of tubules.

If possible entire nephrons have been isolated, as these give information not only as to the size of the segments, but also as to the proportion of them in relation to each other. It is in many cases exceedingly difficult or impossible to avoid breakage, especially in the thin segment, even when the maceration is successful. In such cases it has often been possible to see the thin segment in connection with the other segments, before the breakage. In this case the nephron has been treated as if intact. In other cases the connection between the proximal tubule and the distal parts has been considered sufficiently established if the thick segment is attached to the capsule at its transition into the distal tubule. GRAFFLIN (1939, p. 708) discusses the justness of this procedure. In most kidneys it is easy to ascertain that the thick segment touches and is attached only to the capsule that belongs to the same nephron as the segment itself. Where the cortex is thick and the connective tissue tough it sometimes happens that the thick segment is attached to more than one capsule. Then the above-mentioned procedure cannot be adopted, and this is also impossible when the attachment is lost during maceration or dissection. This occurs especially after injection of the acid according to HUBER.

The measurements have been used as the basis for several calculations. By multiplying the two measured diameters of the capsule an equivalent of the surface of the capsule has been obtained. This might seem too great an approximation, and it might also be assumed that this method is inferior to the one employed by v. MÖLLENDORFF (1922) and PETERS (1928). They measured the diameter parallel to the line of vision also. It seems to me that the accuracy in the measurement of this diameter is so low that the errors introduced thereby probably more than outweigh the theoretical advantages of taking all three dimensions in the calculations. It is also evident that, when the capsules have approximately the same shape, no important error is introduced by assuming that the third diameter is proportional to the other two. If, however, systematic variations in shape occur, this error is of course important. Thus, flattened capsules cannot well be compared with spherical ones.

On analogous reasons it seems unnecessary to measure the thickness of the tubules in more than one plane. The surface equivalent has accordingly been obtained as the product of the length and the mean breadth of the segment. For each proximal tubule connected with its capsule, the ratio of the surface equivalents of the proximal tubule and the capsule has been calculated, and given in the tables under the heading "Ind."

The mean length and thickness of the segments have been obtained as the mean of the measurements. Sometimes these means have been corrected, since in some cases relatively too many long, deep nephrons have been measured. Such corrections are mentioned in the description of the species.

The mean length of the thin segment has sometimes been calculated as follows. After maceration a pyramidal piece, containing a small part of the cortex and the corresponding part of the medulla, is teased from the preparation. This piece should include the whole length of all the loops of the nephrons contained in it. The thickness of the inner zone is measured, and then the innermost part of the inner zone is removed. It is then usually possible to dissect a short segment of each of the loops cut at this level and count the loops. If the thickness of the inner stripe is $a$ mm, and the distance from the zone boundary to the level where the counting has been made is $b$ mm, the minimal length of the thin segments reaching below this level is $a + 2\,b$ mm. The mean length is taken as the average of the longest and the shortest loop cut. Thus the whole inner zone of the piece is gradually removed, and the mean length of the thin segments of the long loops may be calculated. The length of the thin segment of the short loops is as a rule easily measured, and if the relative abundance of short and long loops is known the mean length of the thin segment may be calculated. This method does not give very accurate figures, but its results may be used for purposes of comparison.

When means have been calculated for the proximal tubule and the thick segment their standard error has normally also been calculated. It is doubtful if this is always justifiable. The measurements are often very few, and sometimes the distributions are markedly skew. The standard error has, however, been given, as in any case it gives an idea of the significance of the means. In most cases high, middle, and deep nephrons have been selected and measured in numerical proportions approaching as closely as possible the conditions prevailing in the kidney. This procedure has been applied because the length of the nephrons and their segments is often correlated with their position in the cortex. The mean length of the segments is more exactly estimated in this manner than by the mean of random samples. The standard error of the mean is also affected by this procedure. Below is given an example of how the significance of the mean and the standard error are influenced by the procedure.

The majority of the forms examined shows relatively uniform circumstances. In one case (*Mus flavicollis*, "young male", table 22 a, p. 308) the material is large enough to permit of a statistical analysis. In this specimen small samples (about 9 nephrons) have been taken in such a manner that they have a composition (of high, middle and deep nephrons) comparable to the entire kidney. The proximal tubules in fifteen such samples have been measured. For each sample the mean and the standard error has been calculated. The mean of these means and its standard error has also been calculated, and also the mean of the whole material and its standard error and standard deviation. As is to be expected, the mean of the whole number of primary data, $3.541 \pm 0.045$ mm, and the mean of the group means, $3.532 \pm 0.030$ mm, agree well. The standard error is lower when the mean is calculated from the group means. This indicates that these group means are better estimations of the mean of the whole material than those of random samples would be. This is to be expected, as the groups are selected so as to be representative of the whole material. From the standard deviation of the data in the total material, $\pm 0.529$ mm, the standard error of the means of random samples of nine proximal tubules has been calculated. It is $\pm 0.176$ mm. This standard error is about the same as the average of the standard errors obtained for the selected samples, $\pm 0.169 \pm 0.010$ mm.

Thus the means of samples selected so as to be representative, are better estimates of the true mean than are the means of random samples. As their standard error is as large as that of random samples the means of selected samples are more reliable than their standard error would indicate. As this example is typical of most forms examined it seems that the standard errors calculated may be assumed to indicate the significance of the means as safely as standard errors of the means of random samples.