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THE LOCATION OF RENIN IN THE RABBIT KIDNEY

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Renin was discovered just over sixty years ago (Tigerstedt & Bergman, 1898). It has been shown to be a protease which splits off from a plasma protein a polypeptide, angiotensin (hypertensin, angiotonin), of which the amino-acid constitution is known (Skeggs, Marsh, Kahn & Shumway, 1955; Peart & Elliott, 1956; Skeggs, Lentz, Kahn, Shumway & Woods, 1956) and which has been synthesized (Rittel, Iselin, Kappeler, Riniker & Schwyzer, 1957).

Renin is a normal constituent of mammalian kidney cortex, yet its function in the animal economy, if any, is almost unknown. One of the important facts still missing is a knowledge of the particular cells in the kidney cortex from which renin originates. Friedman & Kaplan (1942) attempted to answer the question by studying the renin content of fish kidneys. Extracts of the glomerular kidney of the freshwater fish, *Cyprinus carpio* and *Ameurus nebulosus*, were pressor in the dog, whereas those from the non-glomerular kidneys of the marine midshipman fish, *Porichthys notatus*, were not. This was taken to indicate that renin was produced in or near the glomeruli. It was shown later, however, that pressor substances could not be obtained from the glomerular kidneys of other marine fish (Friedman & Kaplan, 1942; Bean, 1942). Another study of Kaplan & Friedman (1942) of the renin content of kidney from pig foetuses at various stages of development led them to conclude that the tubules may produce renin. A more direct attack was attempted by Friedman & Kaplan (1943), who produced specific necrosis of the proximal tubules by administration of sodium tartrate to rabbits and correlated the degree of necrosis with a reduction in the renin content of the kidneys. They concluded that the proximal tubules were the site of renin formation. This work has been repeated by Govaerts & Verniory (1948) and Yoshimura & Negishi (1954), who were unable to confirm that necrosis of the proximal tubules by tartrate reduced the renin content of the kidney.

Taquini, Fasciolo & Luna (1950) studied the renin content of successive layers down through the kidney cortex of the cat, dog, ox and pig. They found

that the superficial layers contained more renin, and that in the dog the outermost layer contained no glomeruli. In view of this, they concluded that the source of renin could not be in glomeruli or juxtaglomerular structures.

Cook, Gordon & Peart (1956), using rabbits' kidneys, found most of the renin in the outer half of the cortex but none in the thin subcapsular region which is devoid of glomeruli. They attempted to separate glomeruli from the cortex by picking them from a suspension of fragmented kidney with a micropipette. This took too long and the activity of the extracts was inconstant. It seemed clear that, to get a final answer, a more rapid method with a higher yield was required.

We have devised such a technique, whereby magnetic iron oxide is introduced into the glomeruli which are then selectively removed from a suspension of kidney fragments with an electromagnet. This method has been described in detail (Cook & Pickering, 1958) and will merely be outlined here.

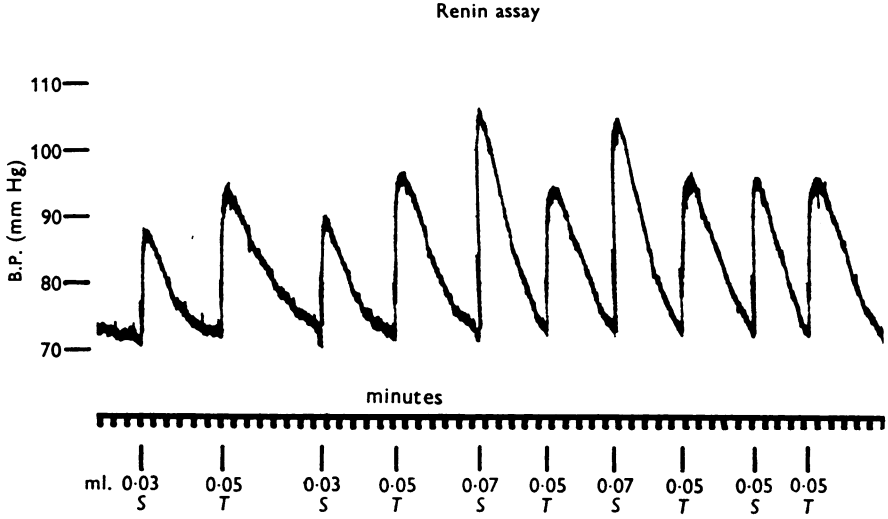
METHODS

Separation of glomeruli from the kidney cortex. A few minutes after intravenous injection of heparin (1000 u.), a healthy rabbit of any age and of either sex was killed by a blow and the renal arteries were quickly cannulated. A suspension of magnetic oxide of iron in gum acacia saline was infused into the kidneys at 120 mm Hg pressure, the renal veins being cut. When about 50 ml. of fluid had been perfused and the kidneys had assumed an even grey colour, they were removed to the cold laboratory (4° C). The kidneys were then sliced and the cortex cut from each slice and pressed through a 150 mesh stainless-steel sieve. The resultant mush was suspended in McEwen's solution (McEwen, 1956), centrifuged slowly and the supernatant fluid discarded. (The choice of McEwen's solution was fortuitous and it was used in all experiments because it proved satisfactory in the first. The more familiar Krebs's solution is also satisfactory.) After so washing twice, the residue was resuspended in McEwen's solution and allowed to flow past an electromagnet, which removed the magnetic glomeruli. This process was repeated until microscopic examination of the magnetic and non-magnetic fractions showed that a satisfactory separation of glomeruli had been achieved (Plate 1). Portions of both fractions were then taken; 0·2 ml. for nitrogen determination, 5 ml. for renin estimation and the remainder for histology. The time taken from excising the kidney to the final division varied between 1 and 3 hr. Renal fragments so prepared and so suspended in McEwen's solution at 4° C showed no diminution of renin content in 6 hr.

Renin assay. The tissue, which had been kept in the deep freeze at −20° C until required, was homogenized, made to a known concentration with saline and centrifuged. The supernatant fluid was then assayed for renin by its pressor effect in the rat anaesthetized with pentobarbital and treated with pentapyrrolidinium, the blood pressure being recorded from the common carotid artery (cf. Peart, 1955). With the exception of the first six experiments (Table 2), each unknown sample was compared with a standard preparation of rabbit renin made by the alcohol method of Pickering & Prinzmetal (1938). Alternate doses of unknown and standard were given and the unknown was bracketed between two doses of standard (Text-fig. 1 is a typical assay). The substance assayed was characterized as renin by the following criteria: (1) the shape and duration of the recorded response were identical with that due to the standard renin; (2) boiling for 5 min destroyed all pressor activity of the extracts; (3) samples, at random, were incubated with rat plasma to produce angiotensin, identified by its pressor response and resistance to boiling. Addition of the magnetic oxide to samples did not reduce the pressor activity of active extracts, nor did it give pressor activity to inactive extracts. While the standard used in this work was prepared in the same way as that of Pickering & Prinzmetal (1938), it cannot be assumed to be identical

with it. The same standard was, however, used throughout this work and internal comparisons are legitimate.

Nitrogen determination. Total nitrogen in 0·2 ml. portions of the tissue suspension was determined in triplicate by a micro-Kjeldahl method of Hawes & Skavinski (1942). A slight modification was introduced in that the apparatus was all glass, and a solution of 4 g boric acid and 10 g NaCl/100 ml. was used to absorb the ammonia, which was titrated with 0·0143 N-H_2SO_4, by means of a micrometer syringe and bromocresol green indicator. The nitrogen estimations were found to be unaffected by the presence of the magnetic iron oxide.



Text-fig. 1. The assay of the renin content of an extract. The pressor effect in the rat of 0·05 ml. of the extract (at *T*) is compared with 0·03, 0·05 and 0·07 ml. of the standard renin (at *S*).

TABLE 1. Nitrogen determinations in samples of tissue suspensions

	Ordinary micro-Kjeldahl method (mg N/10 ml.)	Hawes & Skavinski method (mg N/10 ml.)
1	1·84	1·78
2	1·78	1·81
3	1·87	1·81
Average	1·83	1·80

To test the accuracy of the sampling method, a determination of the nitrogen in 10 ml. of a kidney tissue suspension was made: (*a*) by ordinary micro-Kjeldahl method, with 10 ml. portions; (*b*) by the Hawes & Skavinski (1942), method, with 0·2 ml. portions. Table 1 shows that the methods agree well.

Histology. After samples had been taken for nitrogen and renin estimations, the remaining tissue was fixed in Bouin's fluid for subsequent microscopic investigation of the suspension both intact and after paraffin embedding and sectioning (Plate 3). These examinations have enabled us to estimate the proportions of glomerular and tubular tissue in each preparation. We had hoped to estimate the cells of the juxtaglomerular apparatus too. While we have been able to demonstrate these cells in the attachments of glomeruli so separated, this was so uncertain that we have refrained from estimation.

RESULTS

The magnetic fraction, separated in the way described, consisted almost entirely of glomeruli, most of which were without Bowman's capsule (Plate 1*a*), About 1–2%, however, retained a complete capsule, together with varying lengths of proximal tubule and some tissue at the pole, sometimes including the macula densa. In some well filled glomeruli the oxide extended back into the afferent arteriole (Plate 3*b*), allowing a positive identification of this structure. By pouring through the 150 mesh sieve the magnetic fraction was further separated into glomeruli with attached fragments of other tissue, which remained on top of the sieve, and glomeruli without, or with relatively small, attachments, which went through (Plate 2). Only those glomeruli without large attachments were used in the experiments, unless otherwise stated.

The non-magnetic fraction consisted chiefly of fragmented tubules but contained small fragments of vascular and connective tissue (Plate 1*b*). It looked, in fact, what one would expect of fragmented kidney tissue from which the glomeruli had been removed. In some cases detailed examination showed the presence of a few glomeruli without iron oxide. These and identifiable fragments of glomeruli never amounted to more than about 5% of the tissue and were generally very much less. The glomerular and tubular contents respectively of the magnetic and non-magnetic fractions were determined by spreading a sample of each fraction on a glass slide engraved with a graticule and estimating the areas occupied by these structures and other tissue.

TABLE 2. Contents and pressor activity of magnetic and non-magnetic fractions

Experiment	Magnetic fractions (% non-glomerular contamination)	Non-magnetic fractions (% glomerular contamination)	Pressor activity; ratio of magnetic to non-magnetic fraction (per mg dry tissue weight)
1	2	5	5
2	1	1	6
3	0	1	5
4	1	1	5
5	0	1	7·5 (12·5)*
6†	5	1	10·5 (40)

* Figures in brackets represent ratio after allowance for the oxide in the glomeruli.

† Animal fed on cholesterol diet 1 month.

Table 2 shows the results of six experiments in which the pressor activity per milligram dry weight of glomeruli without attachments was compared with that of the non-magnetic fraction. Experiments 1 to 4 took no account of the iron oxide present in the glomeruli. In Experiments 5 and 6 the oxide was separated magnetically, washed with strong alkali, water, alcohol and ether, then dried to constant weight. The sources of error of this method were

considerable and in subsequent experiments total nitrogen was used as a basis on which to compare the renin content.

In the next series of experiments (Table 3) the cortex was divided by a free-hand cut into an outer and an inner layer of approximately equal thickness, and magnetic and non-magnetic fractions separated from each layer in the ordinary way. The order in which the inner and outer layers were processed was varied systematically in the whole series. Glomeruli from both layers were used, but only tubules from the outer layer were used for comparison because the inner layer could not be guaranteed free from medullary tissue, which does not contain renin. As Table 3 shows, the outer glomeruli always contained more renin per milligram of nitrogen than the inner glomeruli, which in turn contained more than the outer tubules.

TABLE 3. Renin content of glomeruli and tubules (units/mg N)

Outer glomeruli	210	67	64	63	23	10	3·5
Inner glomeruli	53	21	24	19	4	1·9	0·6
Outer tubules	1·2	2·1	0·9	0·4	0·8	1·9	0·3

TABLE 4. Renin content of glomeruli (units/mg N) in different parts of the cortex

	Whole cortex			Outer	Outer	Outer	Inner
With attached fragments	42	22	35·8	150	7·7	47	7·6
Without attached fragments	7·6	3·8	5·3	64	3·5	5·1	1·1

In the preceding experiments the glomeruli with attached fragments of other tissue had always been discarded as impure. Curiosity led us, however, to estimate their renin content, which was found to be very high. A series of experiments was therefore deliberately performed to compare glomeruli with and without attachments. The results are recorded in Table 4 which shows experiments made with the whole cortex, and with its outer and inner layers separately. Whatever the source, the glomeruli with attachments contained more renin per milligram of nitrogen than those without, or with fewer, such fragments.

The large variations in the renin content in different experiments made us wonder whether the method of processing was itself responsible for the variations. To investigate this, both kidneys were injected with iron oxide and one was bisected coronally. The two halves of the one kidney and the whole of the other were processed separately. The results shown in Table 5 demonstrate a remarkably good agreement. The differences in renin content per milligram of nitrogen between glomeruli separated from different rabbits thus probably represent real variations. The range of variation, for example, among inner glomeruli or among outer glomeruli in Table 3 is almost a hundredfold. This is greater than the range found by Pickering, Prinzmetal & Kelsall (1942) on

whole kidney, which varied from 0·2 to 5·6 units/g in 60 normal rabbits. They found the content greater in small than in large rabbits.

The non-magnetic fraction undoubtedly contained renin. This might have been there because renin is located in a structure naturally occurring in it, or because inert material picked up active material from the magnetic fraction during processing. To investigate the latter possibility, we added separated glomeruli to medullary tubules, then separated the mixture into magnetic and non-magnetic fractions in the usual way and usual time, assaying the medullary material before and after. Four experiments were done. Two were purely qualitative but showed quite definitely that a small quantity of renin could be transferred to the non-magnetic fraction. Difficulties were experienced

TABLE 5. Renin content (units/mg N) in portions of kidney of the same animal

	Glomeruli			Tubules		
	Bisected kidney			Bisected kidney		
Rabbit	1st half	2nd half	Opposite kidney	1st half	2nd half	Opposite kidney
1	49	44	—	1·9	2	—
2	46	46	44	1·4	1·8	1·7
3	9	10	10	0·5	0·2	0·2

in a quantitative assay owing to the presence of a depressor substance in the medullary tissue. In subsequent experiments this was removed by dialysing all extracts before assay. Of the two quantitative experiments, one was inconclusive because the extract of the medullary tissue which had been added to and separated from glomeruli gave a peculiar response in the assay rat. The other experiment showed that about one-quarter of the renin in the non-magnetic fraction could be accounted for by transfer from the magnetic fraction during processing. We did not pursue these experiments further because of the doubt as to whether they might not mislead us. Clearly, whatever contains renin can be transferred from the magnetic to the non-magnetic fraction. The experiments would not answer the vital question as to the way it was transferred and, in particular, whether it was through transfer of a specific renin-carrying cell.

DISCUSSION

The observations described in this paper suggest that the cells responsible for the formation of renin are in, or close to, the glomeruli. Weight for weight there is always much more renin in the fraction that is predominantly glomerular than in that predominantly tubular. In considering the kind of structure concerned, two other findings must be taken into account, namely, that the outer glomeruli contain more renin than the inner, and that glomeruli with attached fragments contain more than those without such fragments.

These results admit two possible interpretations, depending upon whether the cells which form renin are conspicuous histologically or not. If they are

conspicuous histologically, the most likely possibilities are cells located near the vascular pole of the glomerulus, namely, the macula densa (Plate 3*a*) and the cells that have been variously described as the juxtaglomerular apparatus and 'polkissen'. Non-fibrillary cells in the walls of the renal arteries and arterioles were first described by Ruyter (1925), particularly at the division of the afferent arterioles into the glomerular capillaries. Goormaghtigh (1932) grouped these non-fibrillary cells of the afferent arteriole, together with neighbouring nerves and the contractile cells, as the juxtaglomerular apparatus. Zimmerman (1933) also described non-fibrillary cells between the vascular pole of the glomerulus and the macula densa of the distal tubule. He named these cells the 'polkissen'. Goormaghtigh & Grimson (1939) consider that the cells of the polkissen form a part of the juxtaglomerular apparatus. Goormaghtigh (1944) believes that the cells of the juxtaglomerular apparatus are secretory because they contain granules which are coloured variously with the trichrome stains. These granular cells are most conspicuous in the juxtaglomerular apparatus of the superficial glomeruli. They are less conspicuous or absent from the deeper glomeruli and they occur occasionally in the media of the afferent arterioles. He has shown that these cells increase in number and in granularity when the renal artery is constricted by Goldblatt's method in the rabbit. During the first 48 hr the granules tend to disappear from the superficial glomeruli. After 8½ hr mitoses are found, and after 24 hr granulations appear in the cells of the arterioles. From 48 hr a strong granular reaction appears in all the juxtaglomerular apparatuses. This reaction persists during at least 6 months, after which it subsides. This evidence has led Goormaghtigh to suppose that these granular cells secrete a vasoactive substance, probably renin. Other observations made in the human subject have led him to similar conclusions. Although Goormaghtigh did not assay his kidneys for renin, this was done in the rabbit by Pickering *et al.* (1942), who found an increase in the renin content of the kidney during the first week after renal artery constriction, but after 2 months or more the renin content was normal.

Goormaghtigh's (1944) observations in the hypertensive rabbit were in general confirmed by Dunihue & Candon (1940) and Dunihue (1941). Goormaghtigh & Grimson (1939) reported hypertrophy of the juxtaglomerular apparatus in renal ischaemia in dogs whose renal arteries had been constricted and who had hypertension of 2–18 months' duration. Kaufman (1948) described hypertrophy and hyperplasia but not increased granularity of the juxtaglomerular apparatus in chronic pyelonephritis and hypertension in man. Graef & Smith (1940), examining kidneys of various species, including dog and man, confirmed the increase of granular cells in renal ischaemia described by Goormaghtigh, but said 'Variation in the appearance of the arteriolar media in normal kidneys is great enough to warrant doubt concerning the significance of hyperplastic changes when observed without evaluation of blood flow'.

Hartroft & Hartroft (1953) showed that in rats a diet low in sodium chloride caused accumulation of granules, while 2% sodium chloride in the drinking water caused degranulation of the juxtaglomerular cells. Deoxycortone acetate also produced degranulation of the cells, an observation confirmed by Dunihue & Robertson (1957). Tobian, Thompson, Twedt & Janacek (1958), counting the juxtaglomerular cells and their granularity by the Hartroft method, found that in the rat constricting one renal artery, the other kidney being intact, led to an increase in granularity in the clipped and a decrease in the unclipped kidney after 10 weeks. A low-salt diet increased the granulation, while deoxycortone acetate and salt caused disappearance of the granules: adrenalectomy doubled the juxtaglomerular index. Recently Tobian, Janacek & Tomboulian (1959) have found a strong correlation between the granulation of the juxtaglomerular cells and extractable renin in the kidneys of rats with experimental hypertension.

These observations together leave little doubt that the juxtaglomerular cells are secretory, that they are affected by constriction of the renal arteries, and suggest that they play some part in the regulation of salt excretion by the kidney, at least in the rat. However, the observations are far from conclusive in showing that the substance secreted is in fact renin. Renin does, however, remain a possibility, particularly in view of the changes in the juxtaglomerular apparatus and in the renal content of renin effected by renal artery constriction, and the fact that in the rat as in the rabbit (Pickering & Prinzmetal, 1940) renin has an action on the renal excretion of sodium and chloride.

If renin is secreted by a cell, or contained in a structure, without conspicuous histological characteristics, the chief possibilities are the cells of the epithelial lining of Bowman's capsule and its underlying basement membrane, and the cells of the glomerular capillaries. Recently Nairn, Fraser & Chadwick (1959) have prepared an anti-serum to purified pig renin and have found that the fluorescent-labelled antibody is most abundantly fixed by the cells and basement membrane of Bowman's capsule and glomerular capillaries. However, these observations again are not conclusive, since the renin was not chemically pure and it is known that antibodies to crude renal extracts are most abundantly located in similar structures (Cruickshank & Hill, 1953; Hill & Cruickshank, 1953).

Our observations are clearly compatible with either of the above hypotheses. Thus a structure at the vascular pole might be found in both fractions but would be more abundant in the magnetic fraction and would be expected to occur more frequently in glomeruli with large attached fragments than in those with small, or no such, fragments. Again, the origin of renin in the juxtaglomerular cells would account for our observation of the relative richness of the superficial glomeruli. Using Masson's trichrome stain (Goormaghtigh, 1944) and Bowie's stain (Hartroft & Hartroft, 1953), we have been able to

demonstrate granular cells in the walls of the afferent arteriole proximal to its branching into the glomerular capillaries in sections of the glomeruli isolated by the magnetic method described. These granules have been easier to demonstrate in the preparations of glomeruli with attached fragments, as would be expected.

Our observations would however be just as easily explained by the origin of renin in the capsule, either in the cells of the epithelium or basement membrane. Thus, when the kidney is pressed through the sieve some glomeruli retain their capsules complete while others lose the parietal layer. These cells no doubt appear in the non-magnetic fraction. Glomeruli with attached fragments mostly have complete capsules. Most of those without fragments have lost the parietal part of the capsule (Plate 2). In this way the greater richness of the glomeruli with fragments could be explained. It will be clear from this discussion that we cannot go further than to say that renin is located in or near to the glomerulus. The exact nature of the cells concerned remains to be determined.

Bing & Wiberg (1958) have recently described observations with a similar purpose to that described here. In the rabbit kidney they too have found the outer non-glomerular zone devoid of renin, while in the glomerular zone there is more per unit volume of tissue in the peripheral cortex than in deeper regions. To locate renin more accurately they used two methods. They found that destruction of the glomerular tufts alone, by a minute thermo-cautery, did not alter the renin content; but if the destruction was more extensive, so that the zone surrounding the tufts was discoloured, the renin content was reduced to about half. They concluded, therefore, that renin was probably located in part of the tubular or in the small vessels adjacent to the glomeruli. This conclusion was fortified by estimations made on glomerular tufts removed from sections by micro-manipulations. These tufts contained no detectable renin while the tissue left after their removal did.

SUMMARY

1. A rapid method for separating glomeruli from other renal tissue has been devised. The glomeruli were filled with magnetic oxide of iron, the kidney fragmented by pressing through a sieve and the glomeruli separated by a magnet from the rest.

2. The magnetic fraction, over 95% of which was glomeruli, always contained more renin per milligram of nitrogen than the non-magnetic fraction, over 95% of which was tubules.

3. The glomeruli of the outer cortex contained more renin per milligram of nitrogen than those of the inner cortex.

4. Glomeruli with attached fragments contained more renin per milligram of nitrogen than those without such fragments. Glomeruli with fragments

differed from those without in having capsules with attached segments of proximal tubule, afferent and efferent arteries and macula densa.

5. It is concluded that renin originates in cells in, or close to, the glomerulus. The most probable are either specialized cells at the vascular pole (the granular cells of the juxtaglomerular apparatus, the cells of the polkissen, or those of the macula densa), or else the epithelial cells of Bowman's capsule and glomerular capillaries. The evidence does not yet allow a decision as to which.

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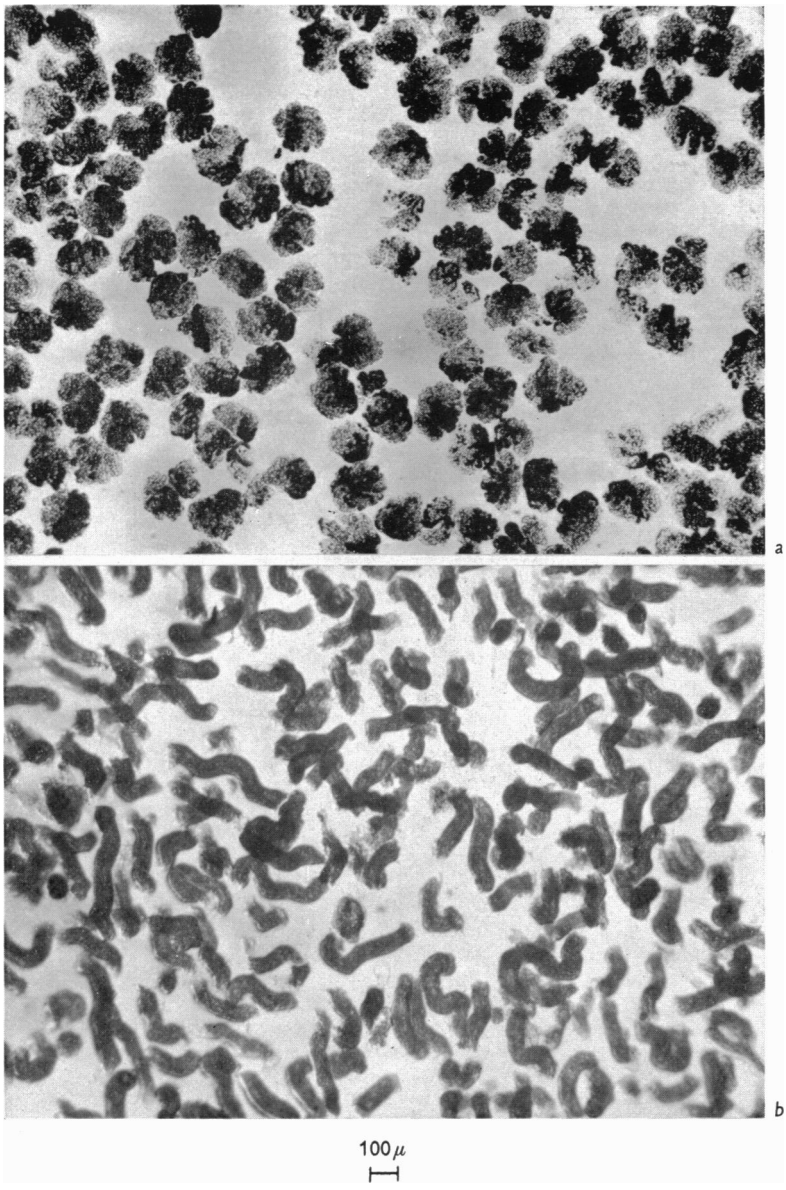
EXPLANATION OF PLATES

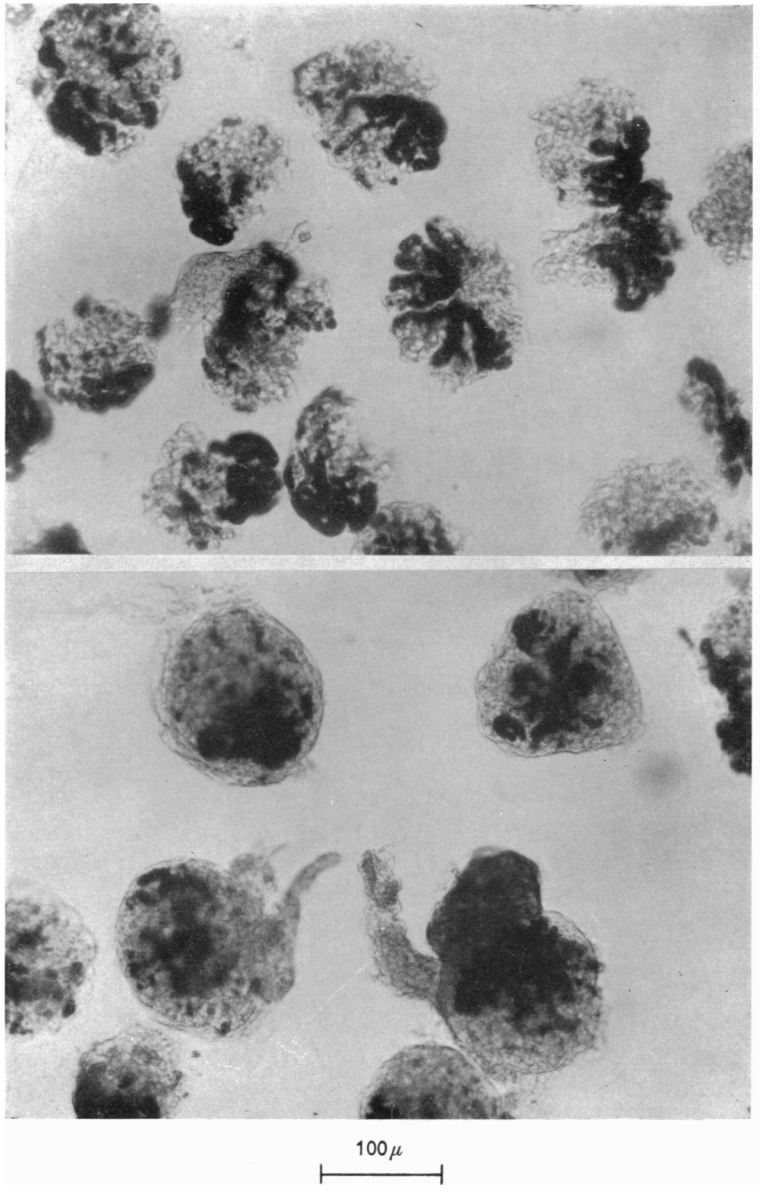
Plate 1. Magnetic (*a*) and non-magnetic (*b*) fractions of kidney.

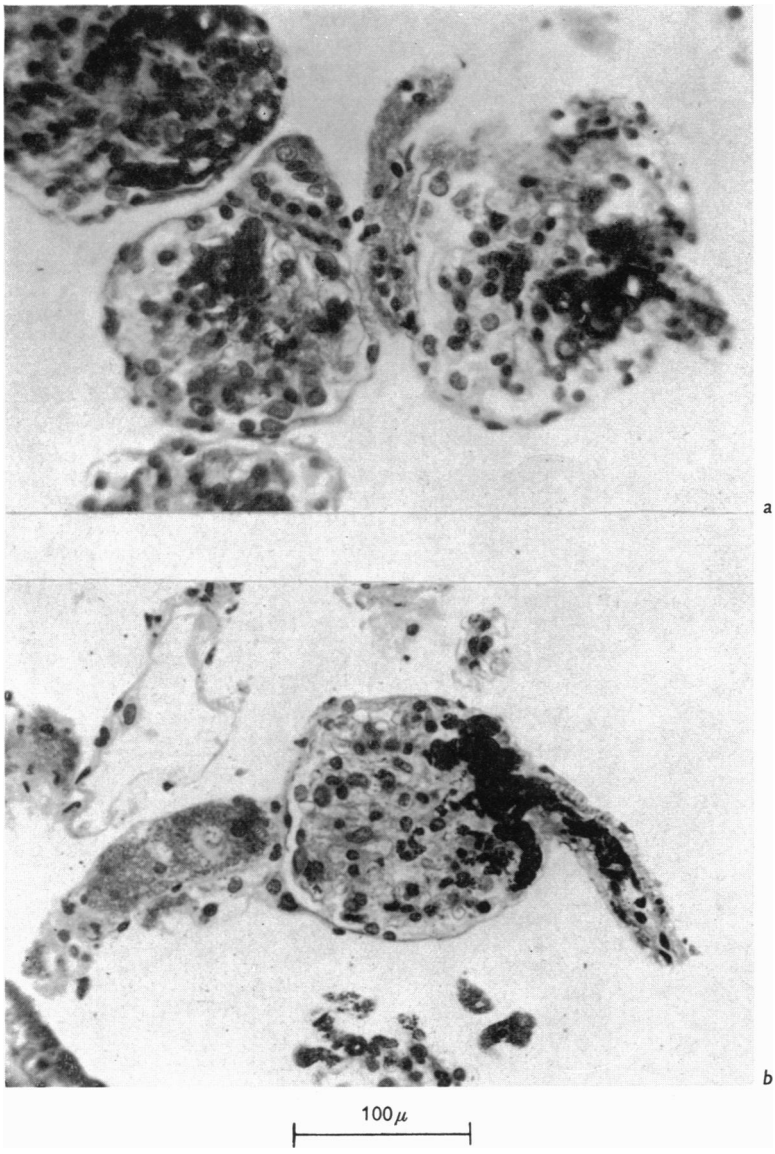
Plate 2. Glomeruli without (*a*) and with (*b*) attached fragments of other tissue.

Plate 3. Masson trichrome stained sections of glomeruli with attachments. (*a*) The macula densa attached to the upper edge of the centre glomerulus; (*b*) a glomerulus with capsule and proximal tubule (to the left) and afferent arteriole (to the right).



(*Facing p.* 536)


a
b
100 μ


a
b
100 μ