# STUDIES CONCERNING THE SITE OF RENIN FORMATION IN THE KIDNEY

## III. The Apparent Site of Renin Formation in the Tubules of the Mesonephros and Metanephros of the Hog Fetus*

By A. KAPLAN, Ph.D., and MEYER FRIEDMAN, M.D.

With the Technical Assistance of Eleanor Williams

*(From the Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, San Francisco)*

Plates 23 and 24

(Received for publication, May 27, 1942)

In the first study (1) of the present series, the inability to detect a pressor substance in the aglomerular kidney of the midshipman fish, a marine variety, while abundant amounts of renin were demonstrated in the glomerular kidney of the catfish and carp, suggested that the site of renin production might be in the glomerular or juxtaglomerular component of the kidney. However, in subsequent studies (2, 3), it was found that no pressor substance, effective in the dog, could be found in the glomerular kidney of several other marine fish (cod, sole, mackerel) investigated in this laboratory. Bean (4), moreover, has recently reported the absence of renin in the kidney of the shark, a marine fish possessing a glomerular kidney. All observations thus point to the conclusion that the kidneys of marine fish, whether glomerular or aglomerular, are probably devoid of a renin-like, pressor substance.

The failure of extracts of marine fish kidneys to exert a pressor effect when injected intravenously into dogs might possibly be due to the species specificity necessary for the reaction between renin and the activator (hypertensinogen?) in plasma as reported by Bean (4) and by Corcoran, Helmer, and Page (5). This possibility is unlikely, however, in view of the fact that extracts of kidneys of fresh water fish exhibit pressor properties typical of renin when injected intravenously into dogs. It should also be noted that Bean (4) was unable to detect the presence of renin in shark kidney by either (*a*) injecting shark kidney extract into other poikilotherms and thus eliminating the effect of species specificity or (*b*) by obtaining hypertensin (angiotonin) after incubation of shark kidney extract with plasma.

In the present report, the results of a study of the renin content and histological structure of the mesonephros and metanephros of the developing hog fetus are given, for it was found that these two kidneys followed entirely different paths of development as the fetus increased in length. The mesonephros of

* Aided by a grant from the Dazian Foundation for Medical Research.

fetuses over 20 mm. in length underwent progressive degeneration and terminal metaplasia, whereas the metanephros continually increased in size and development as gestation approached. During the various periods studied of growth or degeneration of metanephros and mesonephros, the tubules, glomeruli, and renal connective tissue did not participate to an equal extent. This differential proliferation or degeneration made possible a correlation of the renin content of the mesonephros and metanephros of developing hog fetuses with the number, size, and state of the glomeruli and tubules. This correlation has enabled us to perceive that the production of renin in the fetal kidney is associated primarily with tubular, not glomerular, activity or growth. The absence of specialized juxtaglomerular cells in either type of kidney also points to this as the fact.

## *Methods*

*A. Selection and Collection of Material.*—Fetuses of freshly slaughtered hogs were collected and brought to the laboratory for dissection. Because the single metanephros or mesonephros was much too small for individual renin extraction, numerous collections and pooling of both types of kidneys were made within certain length ranges, arbitrarily selected. Thus, the mesonephric tissue of fetuses between 17 to 24, 25 to 49, 50 to 74, and 75 to 99 mm. respectively, were pooled. The metanephra of fetuses between 25 to 49, 50 to 74, 75 to 99, 100 to 199, and 200 to 300 mm. respectively, likewise were pooled.

For the purpose of rapid and facile dissection of the smaller fetuses (under 100 mm.), the fetuses were placed overnight in ice cold acetone and then dissected. The partially dehydrated mesonephros and metanephros were removed, treated with ice cold acetone, ether, and then dried, weighed, and pulverized. The dry powder of each collection was then stored in a desiccator until enough had been accumulated for renin extraction (powdered tissue equivalent to 30 gm. of fresh kidney). The weight of the original, fresh kidneys was calculated from the weight of the dehydrated, defatted kidneys by means of a dehydration factor which was obtained from preliminary observations of the difference in the weights of the fresh kidneys and their weights after the desiccation procedures with acetone and ether.

*B. Preparation and Assay of Renal Extracts.*—The powder equivalent to 30 gm. of fresh kidney was extracted for renin according to the method of Helmer and Page (6), except for several modifications (1). The process was carried to the "Fraction B" stage (6). The extract when finished contained the equivalent of 2 to 3 gm. of fresh kidney per cc. of 0.9 per cent sodium chloride solution.

The physiological assay of the pressor property of any extract was carried out on normal, anesthetized (pentobarbital sodium) dogs according to methods previously described (1). Usually the amount of extract injected at one time contained the equivalent of 5 to 10 gm. of fresh kidney. For the purposes of comparison, however, all increases in blood pressure were expressed as mm. Hg rise per gram of fresh kidney tissue.

For control purposes, 250 gm. of adult hog kidney cortex were dehydrated, defatted, and pulverized as described above, and stored in the desiccator. Then, the equivalent of 35 gm. of fresh kidney cortex was extracted to "Fraction B" stage each time a batch

of fetal kidney powder was prepared. The extract was then tested on dogs in the same manner and at the same time as the fetal kidney extracts. This control procedure served two purposes, namely, (*a*) to check on the consistency of the extraction process and (*b*) to test the assay dog's responsiveness to renin. Employing both criteria as a measure of the adequacy of the assay, the results obtained with fetal extracts prepared at the same time as the control extracts were disregarded if the results with the latter were unsatisfactory. It was found that the extraction of adult hog kidney cortex yielded uniform and consistent results in all experiments but that several dogs were relatively unresponsive to the injection of renin.

### *Description of the Hog Mesonephros*

*Gross Findings.*—The mesonephros was found to be the larger of the two kidneys present in the smallest fetuses examined (17 to 24 mm.) With increasing fetal length, the organ was observed to increase somewhat in size until fetal lengths of 50 mm. or over were reached, after which a decrease associated with obvious degeneration was noted. Fetuses of 100 mm. or over were found to contain no recognizable mesonephric tissue upon gross examination. The arterial and venous circulation of the mesonephros was particularly evident upon its superficial aspect.

*Histological Findings.*—The mesonephric kidney of the smallest fetuses examined (Fig. 1) was found to be composed of tubular epithelium, glomeruli, and a minimum of interstitial tissue.

The tubular epithelium was observed to be almost uniformly cuboidal, and equipped with a brush border. There was a centrally placed, vesicular type of nucleus.

The mesonephra of these young fetuses were found to contain large, well vascularized glomeruli, which usually were crowded together along the external area of the organ with few or none in its central portion. When the mesonephra of larger fetuses were examined however, (Fig. 2) it was found that with advancing age of the fetus, there occurred a differential degeneration and apparent metaplasia (after 50 mm.) of the tubular component, while the glomeruli apparently remained unchanged in number, structure, and function. The tubular degeneration, which was continuous and progressive, was characterized by: (*a*) dilatation, (*b*) loss of the brush border, (*c*) absence of nucleated cells, and (*d*) actual fragmentation. In the mesonephric remnants of fetuses over 75 mm. in length, the remaining viable portion of the tubular component was seen to be invaded by a new type of tissue, with an actual change in the configuration and staining properties of the original tubular epithelium itself.

Although a thorough and repeated search was made for juxtaglomerular cells resembling those described by Goormaghtigh (7), no evidence could be found of these cells in any of the sections of mesonephric tissue, irrespective of the stage of fetal development. Rather, the afferent arteriole as it entered the glomerulus was seen to be sheathed only by its own simple wall, with no evidence of any specialized, granular cells.

In summary then, the histological study of the mesonephros of fetuses between the lengths of 17 and 100 mm. indicated that there was a continuous and differential necrosis of the tubules, with the glomerular component remaining unchanged until metaplasia of the residual mesonephros occurred.

### *Description of the Hog Metanephros*

*Gross Findings.*—The striking characteristic of the metanephros of the smallest fetuses studied (25 to 30 mm.) was the relative paucity of mature tubular epithelium and the relatively heavy concentration of mature and immature glomeruli. The tubular epithelium (Fig. 3) was for the most part poorly differentiated, rarely equipped with a brush border, and appeared basophilic on staining with hematoxylin and eosin. In the interior of the kidney, however, a few mature appearing tubules were seen, together with numerous large, lobulated glomeruli containing nucleated red blood cells. A good proportion of the metanephric tissue, however, was observed to be mesenchymal interstitial tissue. Some collecting duct epithelium was observed.

When the metanephric tissue of older fetuses (30 to 300 mm.) was studied, it was observed that the growth of the kidney was confined chiefly to the tubular portion (Fig. 4), particularly to the proximal convoluted tubules (as now identified by its brush border). In contrast to the tubular growth, there was a progressive diminution in the amount of the mesenchymal tissue and in the concentration of glomeruli as indicated by the number per low power field. As in the smaller fetuses, the principal site of both tubular and glomerular development was found to be in the peripheral sections of the kidney.

In metanephra of fetuses over 100 mm. in length, a rapid growth and extension of collecting duct epithelium was observed, which extended from the beginning pelvis of the kidney and radiated outwards, toward the periphery.

Here again, extensive study of many sections of metanephra taken from fetuses varying from 20 to 300 mm. in length failed to reveal any accumulation of cells in a juxtaglomerular position which could be considered secretory in function. As can be seen in Fig. 5, the afferent glomerular arteriole invariably was found to be devoid of any specialized, granular cells. In view of these observations, it was concluded that the fetal metanephros lacked the juxtaglomerular accumulation of granular cells described by Goormaghtigh (7) in the kidney of the adult rabbit.

Complete transverse sections of metanephra taken from fetuses of varying length were examined for the number of mature glomeruli they contained per low power field (magnification, 100 times). The entire kidney section was divided into areas approximating the diameter of the low power field. The average number of glomeruli per low power field obtained in this manner was averaged with similar results obtained from other counts of metanephric tissue from fetuses of similar size. It was found that the metanephric kidney from fetuses of 25 to 49, 50 to 74, 75 to 99, 100 to 199, and 200 to 300 mm. lengths,

contained an average of 14, 11, 9, 9, and 8 mature glomeruli respectively, per low power field, indicating that the number of glomeruli per gram of kidney tissue decreased with advancing fetal development.

TABLE I

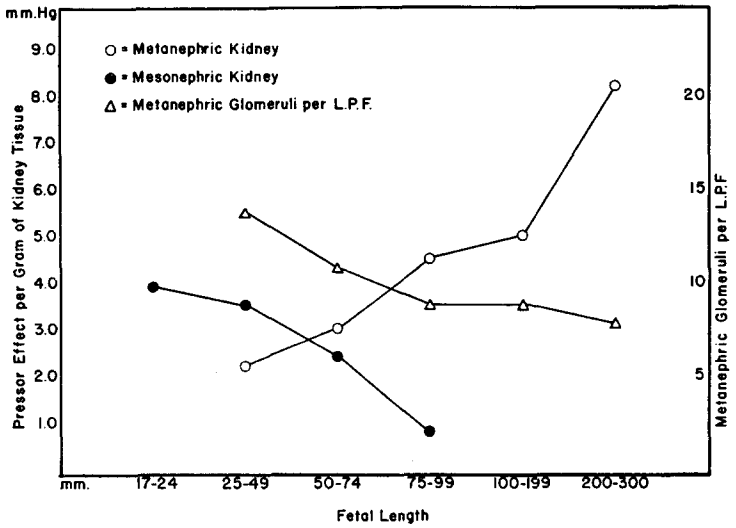
*The Renin Content of Mesonephric Kidney at Various Stages of Fetal Growth*

| Length of fetus | Fetal kidney extract | Recipient dog | Extract injected (*gm.* fresh kidney) | Mean arterial blood pressure | | Pressor effect (Rise per gm. kidney) |
|---|---|---|---|---|---|---|
| | | | | Before injection | After injection of fetal kidney extract | |
| *mm.* | | | | *mm. Hg* | *mm. Hg* | *mm. Hg* |
| 17 to 24 | 21-S | 42 | 5.0 | 144 | 160 | 3.2 |
| | 21-S | 38 | 5.0 | 118 | 146 | 5.6 |
| | 149-S | 12 | 5.0 | 128 | 148 | 4.0 |
| | 149-S | 46 | 5.0 | 126 | 142 | 3.2 |
| | | | | | | Average 4.0 |
| 25 to 49 | 12-S | 0 | 10.4 | 160 | 181 | 2.0 |
| | 12-S | 0 | 8.3 | 130 | 160 | 3.6 |
| | 64-S | 10 | 5.0 | 106 | 126 | 4.0 |
| | 64-S | 38 | 5.0 | 130 | 146 | 3.2 |
| | 64-S | 38 | 5.0 | 140 | 155 | 3.0 |
| | 100-S | 23 | 5.0 | 155 | 178 | 4.6 |
| | 100-S | 45 | 5.0 | 110 | 134 | 4.8 |
| | | | | | | Average 3.6 |
| 50 to 74 | 69-S | 23 | 10.0 | 86 | 115 | 2.9 |
| | 69-S | 19 | 10.0 | 145 | 164 | 1.9 |
| | 101-S | 3 | 10.0 | 123 | 133 | 1.0 |
| | 101-S | 42 | 10.0 | 152 | 170 | 1.8 |
| | 115-S | 10 | 5.0 | 108 | 132 | 4.8 |
| | | | | | | Average 2.5 |
| 75 to 99 | 25-S | 33 | 5.0 | 138 | 143 | 1.0 |
| | 25-S | 42 | 5.0 | 148 | 148 | .0 |
| | 25-S | 42 | 10.0 | 150 | 168 | 1.8 |
| | 25-S | 38 | 5.0 | 150 | 152 | 0.4 |
| | 150-S | 26 | 10.0 | 138 | 148 | 1.0 |
| | 150-S | 10 | 12.0 | 104 | 116 | 1.0 |
| | | | | | | Average 0.86 |

### *The Amount of Pressor Substance in the Mesonephros*

As can be seen in Table I and Text-fig. 1, the mesonephric tissue of the hog fetus contained a pressor substance effective in the dog which decreased in potency per gram of fresh tissue as the length of the fetus increased. Thus, it

was found that two extracts, obtained from the mesonephra of fetuses between 17 and 24 mm. in length when tested four times on four dogs, caused an average rise in blood pressure of 4.0 mm. Hg per gm. of fresh mesonephros. The average rise in blood pressure following the injection of three different extracts obtained from mesonephra of fetuses between 25 and 49 mm. in length was found to be 3.6 mm. Hg per gm. of fresh mesonephros. Three extracts obtained from the mesonephra of fetuses between 50 and 74 mm. in length, tested five times on five dogs, effected an average rise in blood pressure of 2.5 mm. Hg, while the two extracts obtained from the degenerating mesonephra of fetuses




TEXT-FIG. 1. The pressor effect of mesonephric and metanephric extracts from hog fetuses of increasing length upon the dog. Also the relation of metanephric glomeruli per low power field (× 100) to the size of the fetus.

between 75 and 99 mm. in length, effected an average rise in blood pressure of but 0.86 mm. Hg per gm. of fresh mesonephros.

### *The Amount of Pressor Substance in the Metanephros*

All metanephric kidneys contained a pressor substance in good amount, whatever the length of the fetus from which they came. The concentration increased with the age of the fetus as is not the case in the mesonephros. Thus, an extract obtained from metanephra of fetuses from 25 to 49 mm. in length (Text-fig. 1 and Table II), when tested four times on three dogs, effected an average blood pressure rise of but 2.3 mm. Hg per gm. of fresh metanephros, whereas five extracts obtained from the metanephra of fetuses varying from

TABLE II

*The Renin Content of Metanephric Kidney at Various Stages of Fetal Growth*

| Length of fetus | Fetal kidney extract | Recipient dog | Extract injected (gm. fresh kidney) | Mean arterial blood pressure | | Pressor effect (Rise per gm. kidney) |
|---|---|---|---|---|---|---|
| | | | | Before injection | After injection of fetal kidney extract | |
| *mm.* | | | | *mm. Hg* | *mm. Hg* | *mm. Hg* |
| 25 to 49 | 23-T | 2 | 4.9 | 124 | 136 | 2.5 |
| | 23-T | 45 | 4.9 | 128 | 140 | 2.5 |
| | 23-T | 45 | 4.9 | 140 | 150 | 2.0 |
| | 23-T | 38 | 4.9 | 132 | 142 | 2.0 |
| | | | | | | Average 2.3 |
| 50 to 74 | 43-T | 0 | 15.9 | 132 | 154 | 1.4 |
| | 91-T | 3 | 5.0 | 112 | 126 | 2.8 |
| | 91-T | 42 | 5.0 | 154 | 166 | 2.4 |
| | 50-T | 75 | 7.8 | 145 | 165 | 2.6 |
| | 65-T | 21 | 10.0 | 140 | 164 | 2.4 |
| | 115-T | 4 | 5.0 | 148 | 170 | 4.4 |
| | | | | | | Average 3.1 |
| 75 to 99 | 1 | 17 | 4.4 | 125 | 155 | 6.8 |
| | 1 | 16 | 5.5 | 120 | 138 | 3.3 |
| | 1 | 18 | 8.8 | 130 | 180 | 5.7 |
| | X-6 | 9 | 10.0 | 156 | 194 | 3.8 |
| | 66-T | 9 | 10.0 | 130 | 166 | 3.6 |
| | | | | | | Average 4.6 |
| 100 to 199 | 6 | 7 | 5.5 | 135 | 169 | 6.1 |
| | 22 | 13 | 5.5 | 122 | 148 | 4.7 |
| | 22 | 14 | 5.5 | 140 | 165 | 4.6 |
| | 94-T | 3 | 5.0 | 94 | 126 | 6.4 |
| | 147-T | 24 | 5.0 | 125 | 144 | 3.8 |
| | 147-T | 00 | 5.0 | 150 | 170 | 4.0 |
| | 147-T | 0 | 5.0 | 148 | 187 | 7.8 |
| | 147-T | 26 | 5.0 | 127 | 151 | 4.8 |
| | 155-T | 94 | 5.0 | 140 | 160 | 4.0 |
| | | | | | | Average 5.1 |
| 200 to 300 | 95-T | 3 | 5.0 | 123 | 143 | 4.0 |
| | 95-T | 3 | 5.0 | 128 | 148 | 4.0 |
| | 95-T | 42 | 5.0 | 142 | 178 | 7.2 |
| | 148-T | 10 | 5.0 | 130 | 190 | 12.0 |
| | 148-T | 38 | 2.5 | 134 | 164 | 12.0 |
| | 142-T | 26 | 5.0 | 123 | 175 | 10.4 |
| | 142-T | 24 | 5.0 | 118 | 166 | 9.6 |
| | 142-T | 94 | 5.0 | 137 | 175 | 7.6 |
| | X-T | 94 | 5.0 | 123 | 158 | 7.0 |
| | X-T | 26 | 5.0 | 136 | 188 | 10.4 |
| | X-T | 24 | 5.0 | 128 | 180 | 10.4 |
| | 160-A | 26 | 5.0 | 114 | 190 | 15.2 |
| | 160-A | 26 | 5.0 | 132 | 172 | 8.0 |
| | 160-B | 21 | 5.0 | 148 | 176 | 5.6 |
| | 160-B | 46 | 5.0 | 130 | 164 | 6.8 |
| | 171-T | 12 | 5.0 | 138 | 172 | 6.8 |
| | 171-T | 26 | 3.2 | 134 | 150 | 5.0 |
| | | | | | | Average 8.3 |

50 to 74 mm. in length, when tested six times on six dogs, effected an average blood pressure rise of 3.1 mm. Hg per gm. of fresh tissue. Further, it was found that three extracts obtained from the metanephra of fetuses from 75 to 99 mm. in length caused an average rise of 4.6 mm. of Hg per gm. of fresh metanephros in the blood pressure of the four dogs tested. This progressive increase in the concentration of pressor substance continued with the growth of the fetus. Five extracts obtained from the metanephra of fetuses from 100 to 199 mm. in length were tested on nine dogs and it was observed that a rise in blood pressure of 5.1 mm. Hg per gm. of fresh metanephros was the average result. Finally, the average rise in blood pressure obtained by the injection of 10 dogs with 17 injections of seven different extracts obtained from the metanephra of fetuses varying from 200 to 300 mm. was 8.3 mm. Hg per gm. of fresh metanephros. This latter potency closely approached that of the adult hog kidney cortex, which was found to be 8.8 mm. Hg per gm. of fresh kidney cortex.

*The Identification of the Pressor Substance Found in Fetal Renal Tissue As Renin*

The pressor substance found in both the mesonephric and metanephric tissue of the developing fetus appeared to be identical with renin, whatever the length of the fetus. Extracts obtained from mesonephric tissue of fetuses varying from 17 to 75 mm. in length and from metanephra of fetuses from 25 to 300 mm. in length exhibited a pressor effect which, (*a*) was uniformly slow in beginning but lasted over 10 minutes, (*b*) was not inhibited by the prior intravenous administration of cocaine, and (*c*) was abolished by the prior establishment of a tachyphylaxis to known hog renin in the recipient dog. Conversely, it was established that dogs which had received repeated injections of these fetal extracts became tachyphylactic to further injections of fetal extracts and to injections of known hog renin.

## DISCUSSION

The degeneration of the mesonephros and the growth of the metanephros in the developing hog fetus were found to be characterized by differential degeneration of the tubular component in the former and its growth in the latter type of kidney. The number of glomeruli of the mesonephros remained unchanged despite the tubular decay, whereas those of the metanephros decreased per unit area as tubular proliferation went on. Furthermore, the amount of renin extracted from these two types of kidney, was found to decrease with tubular necrosis and to increase with tubular hyperplasia, despite the condition or number of functioning glomeruli present. Thus, the effective renin content per gram of tissue decreased nearly fourfold in the degenerating mesonephros at the same time that the number and function of glomeruli did not change, and conversely the renin content per gram of tissue increased nearly fourfold in the developing metanephros at the same time that the number of glomeruli

per unit area (and presumably per unit weight) decreased 40 per cent. This indicated clearly that the production of renin was independent of the arteriologlomerular component of the fetal mammalian kidney and varied directly with tubular mass.

Since the results of our present studies indicate that the tubular epithelium of both mesonephros and metanephros is responsible for the formation of renin, it is interesting to speculate upon which section of the tubule is directly concerned. The presence of renin in both the metanephros and mesonephros and the absence of the loop of Henle and extensive collecting duct epithelium in the latter tissue suggest that these portions of the tubules are not responsible for renin production. Eliminating the loop of Henle and collecting duct epithelium by this consideration, only the convoluted tubular epithelium remains as the probable site of formation of renin. It should be noted that it was precisely this type of epithelium which was observed to increase differentially in the growing metanephros. At this time, however, it is still uncertain which portion of the convoluted tubules, the proximal or distal or both, is involved in the formation of renin.

## CONCLUSIONS

1. Renin was found in both the mesonephric and metanephric kidneys of the smallest hog fetuses examined. These were from 17 to 24 mm. in length in the case of the former, and 25 to 49 mm. in that of the latter.

2. No evidence was found in either type of kidney of juxtaglomerular cells described by Goormaghtigh as the probable site of renin formation.

3. The renin content in both the mesonephros and the metanephros was found to be independent of its arteriologlomerular component but directly dependent upon the number, size, and functional state of the tubular component. It increased in amount with increasing tubular proliferation during the course of embryonic development, and decreased with the progressive tubular atrophy and degeneration incident thereto.

4. The site of renin formation is discussed.

The authors wish to express their thanks to Eleanor Kruger and Dr. J. Sugarman for valuable assistance.

## BIBLIOGRAPHY

1. Friedman, M., and Kaplan, A., *J. Exp. Med.*, 1942, **75**, 127.
2. Friedman, M., and Kaplan, A., *Proc. Soc. Exp. Biol. and Med.*, 1942, **50**, 199.
3. Friedman, M., and Kaplan, A., unpublished observations.
4. Bean, J. W., *Fed. Proc.*, 1942, **1**, No. 1, pt. 2, 6.
5. Corcoran, A. C., Helmer, O. M., and Page, I. H., *Fed. Proc.*, 1942, **1**, No. 1, pt. 2, 17.
6. Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.
7. Goormaghtigh, N., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 688.

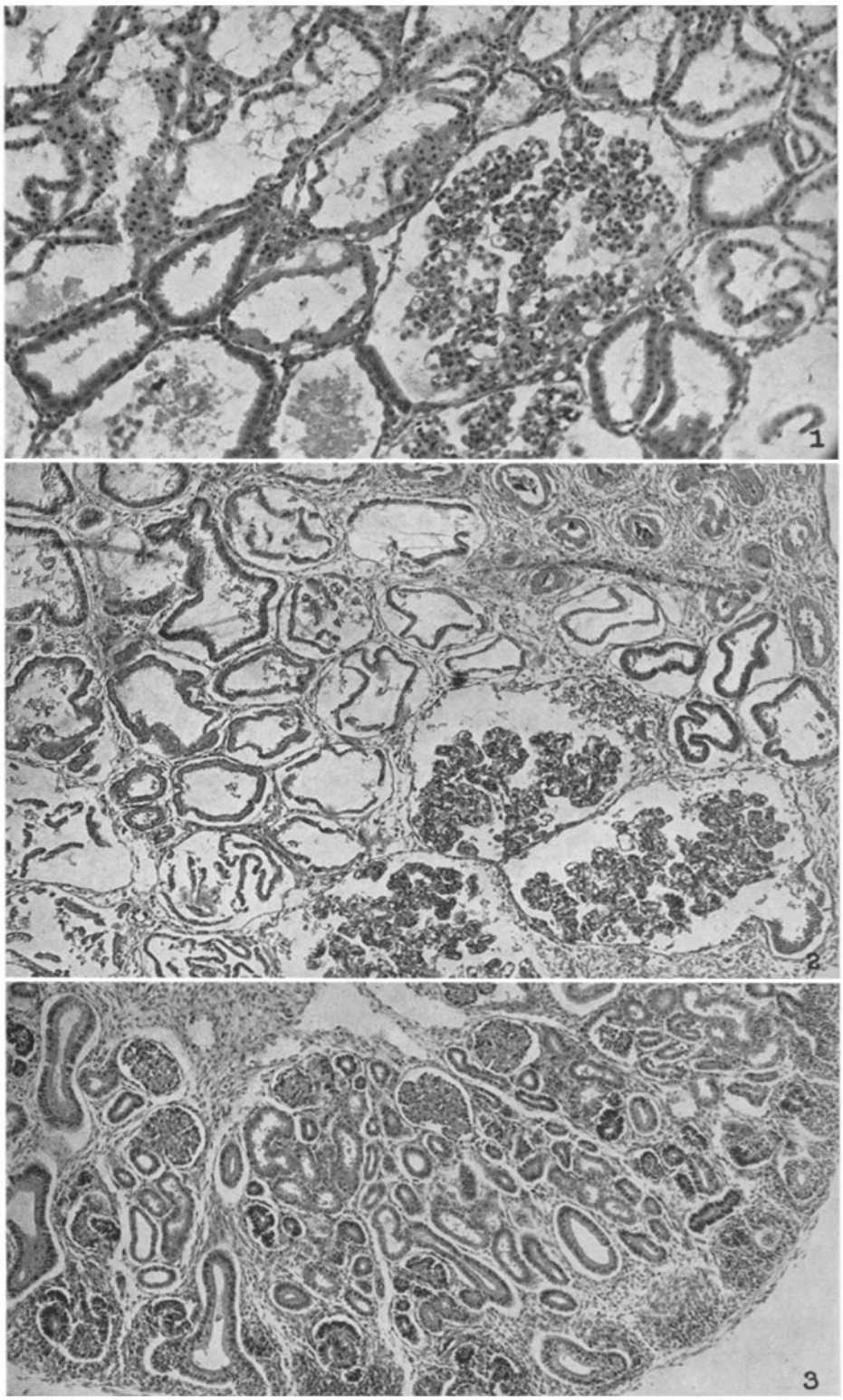
## EXPLANATION OF PLATES

### PLATE 23

FIG. 1. The mesonephros of a hog fetus, 20 mm. in length. Note the large glomerulus which contains nucleated red blood cells. The tubular epithelium appears in good condition and of a cuboidal type. Compare tubules with those shown in Fig. 2. Hematoxylin and eosin. × 200.

FIG. 2. The mesonephros of a hog fetus, 66 mm. in length. In this section, necrotic tubular epithelium, intact, red-blood-cell-containing glomeruli, and invading epigenitalis (upper right) can be seen. The glomeruli do not disappear until all tubular epithelium has been replaced by this latter type of tissue. Hematoxylin and eosin. × 100.

FIG. 3. The metanephros of a hog fetus, 26 mm. in length. Note the large number of glomeruli, both mature and immature, as compared with the immature tubular epithelium widely separated by mesenchymal tissue. Collecting duct epithelium can also be seen. Hematoxylin and eosin. × 100.

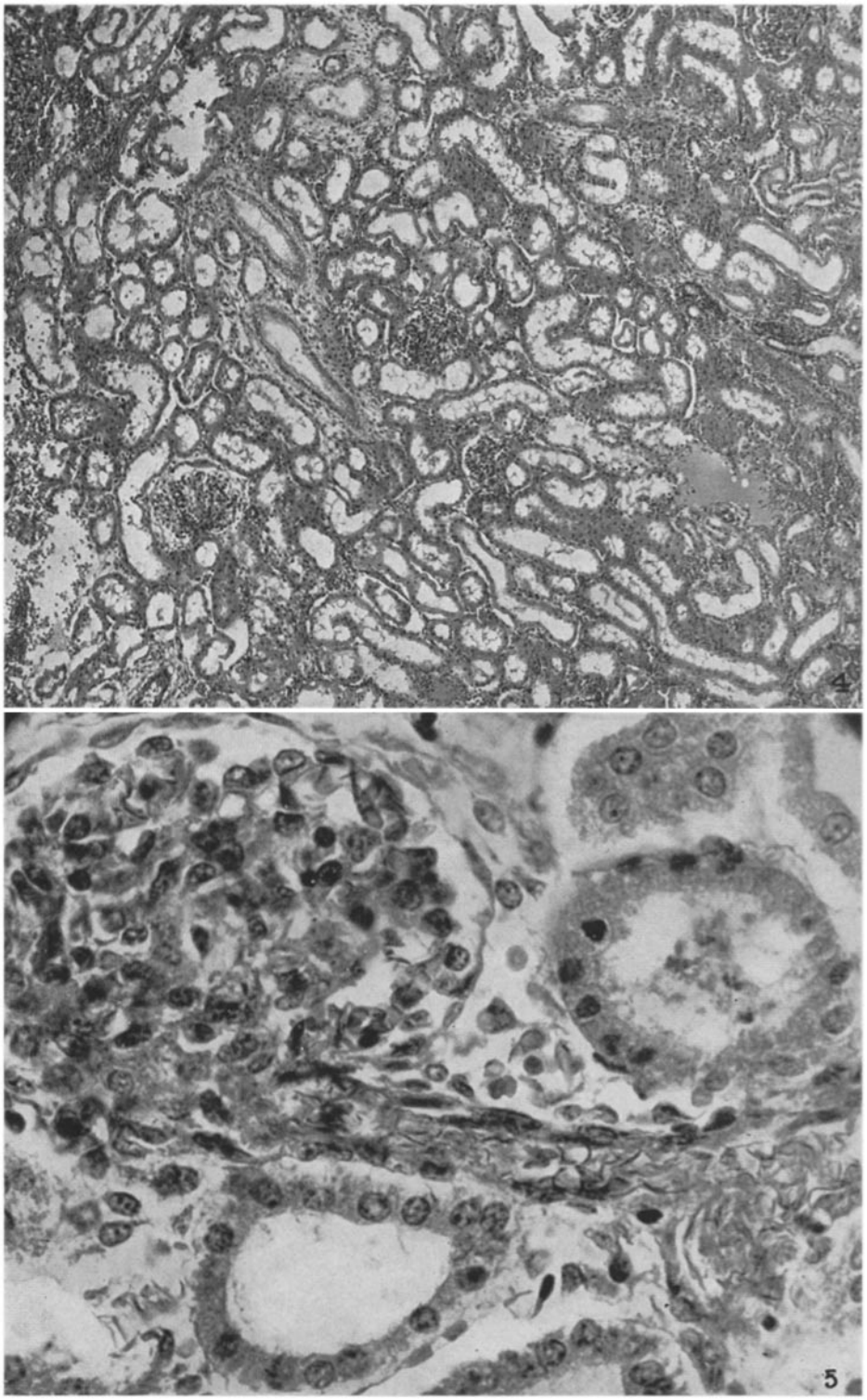



(Kaplan and Friedman: Site of renin formation in kidney. III)

PLATE 24

FIG. 4. The metanephros of a hog fetus, 175 mm. in length. Note the abundance of mature appearing tubular epithelium and the decrease in mesenchymal tissue and glomeruli in this section. Hematoxylin and eosin. × 100.

FIG. 5. The glomerular afferent arteriole in the metanephros of a hog fetus, 66 mm. in length. It is seen to be devoid of any type of cell other than the simple, flat cells composing its wall. Red blood cells can be seen both in the arteriole and in the glomerulus. Hematoxylin and eosin. × 400.




(Kaplan and Friedman: Site of renin formation in kidney. III)