

## STUDIES CONCERNING THE SITE OF RENIN FORMATION IN THE KIDNEY

### I. THE ABSENCE OF RENIN IN THE AGLOMERULAR KIDNEY OF THE MIDSHIPMAN FISH

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#### PLATE 1

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The discovery by Tigerstedt and Bergmann (1) of the pressor substance, renin, in the kidney of the rabbit has been amply confirmed. This substance, moreover, has been found to be present in the kidney of every mammal thus far investigated (2-6). The site of renin production or concentration in the kidney is unknown. Goormaghtigh (7-9), on the basis of histological studies of normal and hypertensive kidneys of rabbits and dogs, suggested that renin is secreted by the juxtaglomerular apparatus. On the other hand, Weeks and his associates (10), from their studies concerning the surgical reduction of blood pressure in hypertensive dogs, suggested that the tubule of the kidney is the site of formation of the pressor substance(s). The physiological interdependence of both the glomerular and the tubular vasculature of the mammalian kidney makes the separate assay of either system for renin content difficult. However, the reported absence of glomeruli and significant arterial circulation in the kidney of the toadfish (11) suggested that renin assays of both glomerular and aglomerular fish kidneys might provide a unique opportunity for the determination of the intrarenal site of renin production.

In the present communication, the results of such a study are reported.

#### *Description of Experimental Material*

*A. The Midshipman Fish (Batrachoididae, Porichthys notatus).*—The midshipman fish was studied because it was suggested to us by Dr. Homer W. Smith that it might have an aglomerular kidney, as both the midshipman fish and the toadfish belong to the same family (Batrachoididae). Careful histological investigation proved his suggestion to be correct.

The midshipman is a marine fish commonly found along the coast of northern California. It is about one foot in length and has three peculiar and identifying characteristics: (1) when in shallow water it emits a low pitched murmur when irritated or disturbed, (2) it has four body lines consisting of

minute yellow-white circular areas running along its ventral surface, the inner two lines joining each other in a wide circular design, and (3) it has a horny spine at the base of the anterolateral fin and a midline dorsal spine at the anterior termination of the dorsal fin. The small circular areas become luminescent at a high pH.

The kidney's gross anatomical structure is identical with the kidney of the toadfish as described by Marshall (11) except that no renal arterial circulation and no midline fusion of the separate kidneys were discerned. Each kidney weighed about 0.5 gm. On histological examination (Fig. 1) with the aid of Mallory's triple stain, the kidney parenchyma was observed to be composed of two types of cells,—(1) lymphoid tissue cells and (2) tubular epithelium cells. No glomeruli were observed, even on serial section of the entire kidney. The tubular epithelium is of one type, consisting of cells which are cuboidal, equipped with a brush border and a nucleus situated proximally in relation to the tubular lumen.

*B. The Carp (Cyprinidae, Cyprinus carpio).*—The kidney of the carp has been described both by Marshall and Smith (12) and by Moore (13). This kidney receives its arterial blood from the metameric arteries arising from the aorta. The glomeruli (Fig. 2) are quite numerous, large, and well lobulated. At the entrance of the arteriole into the glomerulus, an occasional concentration of cells around the arteriole was observed. The nucleus of these cells is vesicular and occasionally surrounded by a halo as described by Kaufmann (14). However, no granules were seen with the Masson trichrome stain, and the exact significance of these cells could not be determined by us. The tubular epithelium appears to be of one type and a brush border was present.

*C. The Catfish (Ameiuridae, Ameiurus nebulosus).*—The kidney of this fish also has been described by the previously mentioned investigators (12, 13). Its vasculature and histological structure were observed to be essentially the same as those of the carp kidney. However, the glomeruli did not appear to be as well vascularized and there were fewer juxtaglomerular groups of cells observed in this species of fish.

*D. The Hog.*—The kidney cortex of the hog was used solely for control purposes. The vasculature, glomeruli, and tubular epithelium of the hog kidney were observed to be essentially similar to those of the human kidney.

#### *Methods of Extraction and Assay*

The extraction of the various types of kidney tissue for renin content was performed with slight modifications according to the method described by Helmer and Page (3).

In brief, 25 to 50 gm. of finely ground, fresh or refrigerated (frozen in this laboratory and stored at  $-40^{\circ}$  or  $-6^{\circ}$ ) kidneys were dehydrated and defatted by two extractions with ice cold acetone and two extractions with ice cold ether at  $0^{\circ}\text{C}$ . The tissue residue was dried in air and pulverized by grinding with sand in a mortar.

Crude extracts were prepared by extracting these powders with three separate portions of 2 per cent sodium chloride solution for 1 hour each at 0°C. After centrifuging, the extracts were combined and poured through a coarse filter paper. A total of 1.5 cc. of 2 per cent saline solution per gram of fresh kidney was employed, but the usual yield after centrifuging and filtering was approximately 1 cc. per gm. of tissue. The crude saline extracts were stored at 0°C. until their assay, which took place between 1 and 20 hours after their preparation. The pH was adjusted to 7.0 with sodium hydroxide just prior to their injection into dogs.

In certain experiments, the extracts were partially purified and concentrated by extending the process to the stage of "Fraction B" of Helmer and Page (3). By this procedure, inert protein was eliminated by adjusting the pH of the crude saline extract to 4.5 with acetic acid. After centrifuging, additional inert material was precipitated by bringing the supernatant fluid to 1 M potassium phosphate concentration (pH 6.5). The mixture was centrifuged and the supernatant fluid adjusted to a concentration of 2 M potassium phosphate solution. After filtering, the residue was dissolved in 6 to 10 cc. of 0.9 per cent sodium chloride solution (Fraction B). All procedures except filtration and centrifugation were carried out at 0°C.

Several portions of the glomerular kidney were refrigerated at -40°C. for 14 days because of the difficulty in obtaining sufficient material for complete assay experiments. Control storage of hog kidney and carp kidney, at -40°C. and -6°C., indicated that no significant diminution of the renin content occurred at these temperatures during this period of time.

Five separate extractions of the midshipman fish kidney (A, B, C, D, and E) were performed and the five extracts were tested on three nephrectomized, anesthetized (pentobarbital sodium) dogs. The pressure was obtained by the cannulation and connection of the left femoral artery to a mercury manometer. The introduction of hog kidney extract equivalent to 5 gm. of the fresh material into each dog after the injection of glomerular kidney extract was found to cause a prolonged pressor effect of over 30 mm. of Hg in every dog.

Four separate extractions of the carp kidney (A, B, C, and D) were performed, and the four extracts were tested on five dogs, three of which were anesthetized but not nephrectomized. One extraction of the catfish kidney (A) was performed and tested on two normal anesthetized dogs.

Two separate extractions of hog kidney were performed and tested on two anesthetized dogs (one dog was normal). Also, previously tested hog extracts were used for the induction of tachyphylaxis in several experiments.

#### RESULTS

Despite the fact that five separate extracts of varying quantities of the fresh and refrigerated glomerular kidneys of the midshipman fish were prepared and tested, none of them were observed to exert a pressor effect upon the nephrectomized, anesthetized dog (Table I and Text-fig. 1). The crude kidney extracts, however, exerted a slight depressor effect on injection.

Each of the five extracts of the glomerular kidney of the carp was observed to exert a preliminary depressor and then a prolonged pressor effect upon the

anesthetized dog. Greater effects were obtained when the dog was nephrectomized prior to the administration of the extract. It should be noted (Table I) that a pressor effect was even obtained with an extract equivalent to as little as 4.5 gm. of fresh carp kidney. Furthermore, it was observed that after the induction of renin tachyphylaxis by the administration of hog renin

TABLE I  
*The Pressor Effect of Extracts of the Glomerular and Agglomerular Kidney*

Test animal	Type of kidney extracted	Amount of kidney tissue extracted	Pressor response	Duration of pressor effect
		gm.	mm. Hg <sup>§</sup>	min.
Dog 1.....	Midshipman fish (A)*	25.0	0	—
Dog 1.....	“ “ (B)	32.0	0	—
Dog 2.....	“ “ (C)	10.2	0	—
Dog 3.....	“ “ (C)	21.4	0	—
Dog 2.....	“ “ (D)‡	8.8	0	—
Dog 3.....	“ “ (D)‡	20.2	0	—
Dog 3.....	“ “ (E)‡	7.5	0	—
Dog 2.....	Carp (A)	9.9	88	Over 28
Dog 3.....	“ (A)	4.5	30	“ 6
Dog 4§.....	“ (B)	8.0	28	“ 7
Dog 5§.....	“ (C)*	20.0	20	“ 7
Dog 6§.....	“ (D)	8.0	48	“ 11
Dog 7§.....	Catfish (A)	5.0	20	“ 10
Dog 8§.....	“ (A)	10	40	“ 8
Dog 2.....	Hog (A)**	5.0	60	“ 50
Dog 9§.....	“ (B)	5.0	60	“ 9

\* Extraction carried to "Fraction B" stage.

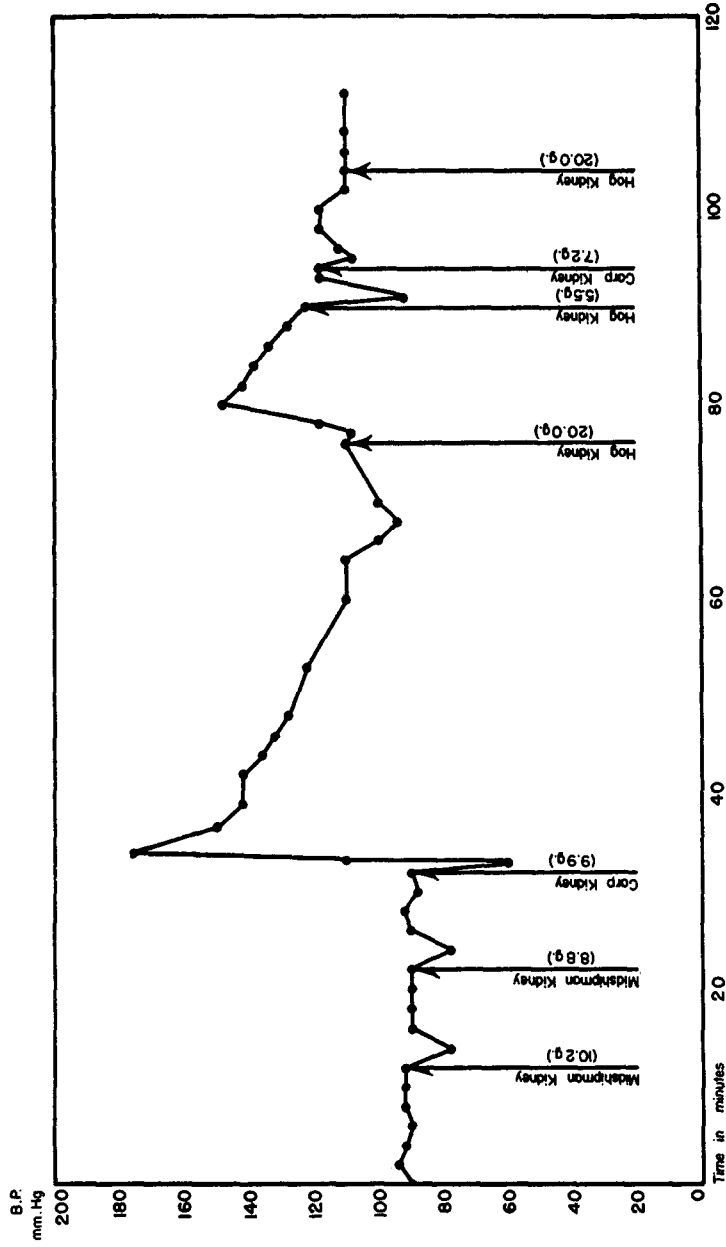
‡ Stored at  $-40^{\circ}\text{C}$ . for 19 days.

§ Dog not nephrectomized.

|| Stored at  $-6^{\circ}\text{C}$ . for 12 days.

\*\* Stored at  $-40^{\circ}\text{C}$ . for 14 days.

(Text-fig. 1), the carp kidney extract failed to exert a pressor effect. In one experiment also, the repeated administration of carp kidney extracts (each extract equivalent to 8.0 gm. of fresh kidney) was followed by a typical renin tachyphylaxis. In view of the fact that (1) carp kidney extract exerts a prolonged pressor effect typical of renin, (2) is capable of producing renin tachyphylaxis, and (3) is incapable of producing a rise in the blood pressure of a dog after the prior induction of renin tachyphylaxis with hog renin, it appears clear that the pressor activity of the carp kidney is due to its renin content.



TEXT-FIG. 1. The pressor effect of aglomerular and glomerular fish kidney extracts upon the nephrectomized dog. Also the effect of fish glomerular kidney extract after the induction of hog renin tachyphylaxis.

The refrigeration of carp kidney for 14 days at  $-6^{\circ}\text{C}$ . was not observed to diminish notably its renin content.

The extract obtained from 5.0 gm. of the glomerular kidney of the catfish also was observed (Table I) to exert a prolonged pressor effect (typical of renin), upon a normal anesthetized dog.

The administration of an extract equivalent to 5 gm. of fresh hog's kidney was found to exert a prolonged and marked pressor effect when given to a normal anesthetized dog (Table I). Furthermore, refrigeration of hog kidney at  $-40^{\circ}\text{C}$ . for 14 days did not destroy its renin content.

#### DISCUSSION

The foregoing observations indicate that not only the kidney of the hog, but also the glomerular kidney of the catfish and carp contain renin. However, repeated assays of the aglomerular kidney of the midshipman fish failed to reveal the presence of any type of pressor substance. Thus, although a strongly pressor extract could be obtained from as little as 4.5 gm. of fresh carp kidney, a similar assay performed upon 32 gm. of fresh midshipman kidney failed to reveal the presence of a pressor substance. In view of these findings, it appears clear that the aglomerular kidney of the latter fish contains no detectable renin.

The presence of renin in the glomerular kidneys of both the carp and catfish suggests strongly that the absence of renin in the aglomerular kidney of the midshipman fish is due primarily to the fact that the kidney of this last species lacks the arterial vasculature and glomeruli found in the two kidneys first mentioned. It strongly suggests, too, that the site of renin manufacture or concentration is not in the tubules of the mammalian kidney.

The presence of a juxtaglomerular accumulation of cells in the kidneys of both the carp and catfish has been described. No evidence of the possible endocrine activity of these cells, however, could be detected from our limited histological studies.

#### SUMMARY

1. The absence of glomeruli in the kidney of the midshipman fish (*Porichthys notatus*) is reported.
2. The detection of renin in the glomerular kidney of the carp, the catfish, and the hog, and the apparent absence of this substance in the aglomerular kidney of the midshipman fish suggest that the tubular portion of the mammalian kidney does not produce or store renin.

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was given to us by Dr. Robert C. Miller of the California Academy of Sciences and by Dr. Rolf L. Bolin of the Hopkins Marine Station.

We were aided in the histological study of the fish kidneys by Dr. G. Rusk of the Mount Zion Hospital.

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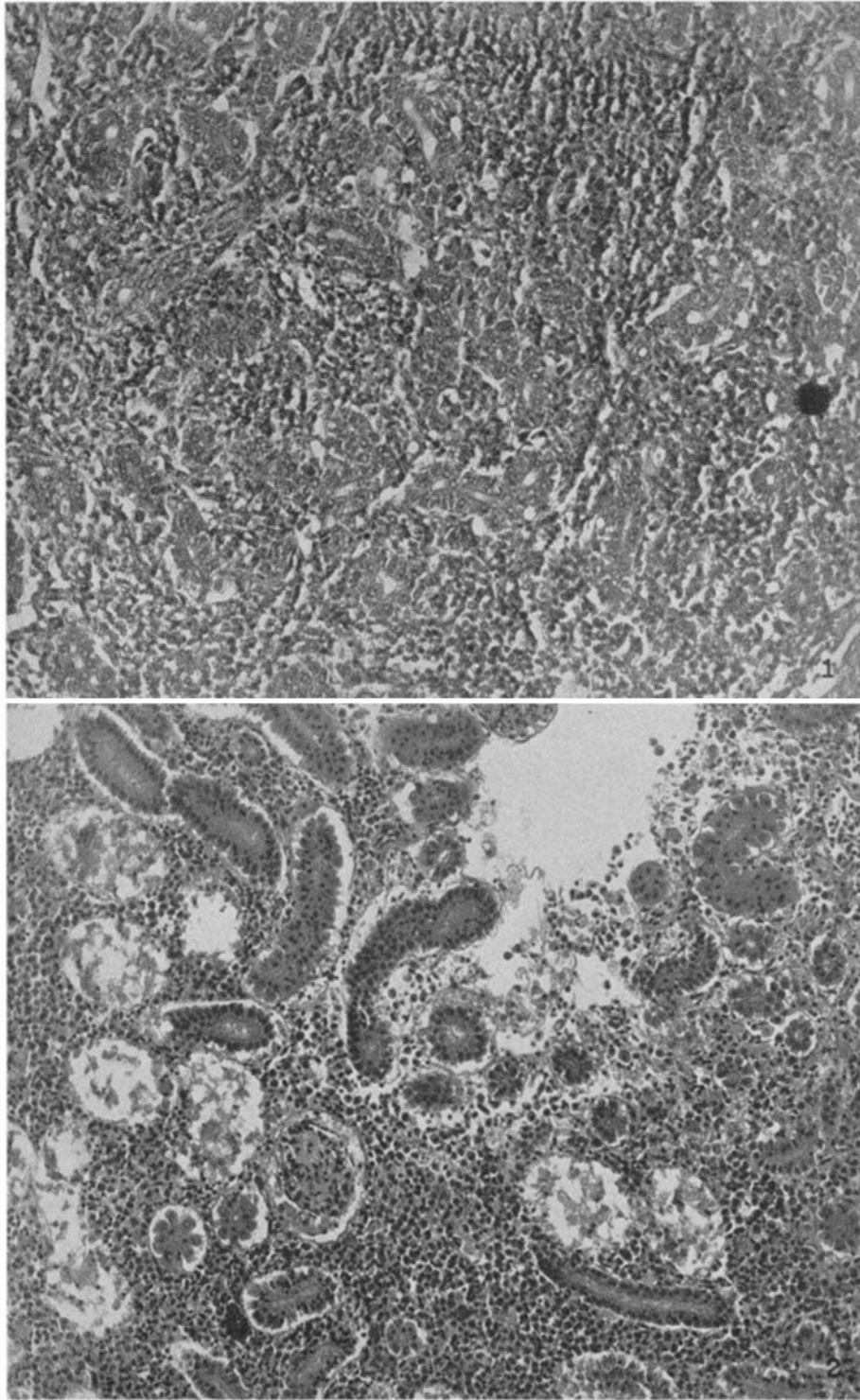
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## EXPLANATION OF PLATE 1

FIG. 1. The aglomerular kidney of the midshipman fish. Note the absence of glomeruli, and the presence of an abundant round cell stroma between the tubular constituents. Mallory's triple stain.  $\times 200$ .

FIG. 2. The glomerular kidney of the carp. There is an abundant stroma composed of red and white blood cells. Note the large and well developed glomerulus. Mallory's triple stain.  $\times 200$ .





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