CHAPTER X

HISTOCHEMICAL CHANGES IN THE KIDNEY OF VAGOTOMIZED AND CHEMICALLY SYMPATHECTOMIZED BLUE ROCK PIGEON (*COLUMBA LIVIA*)

The nerve supply of the kidney appears to have been poorly studied. Mauger (1941) described the extrinsic innervation of the kidney coming from the renal plexus, lying at the origin and interlacing the renal arteries, most of the nerves going to the kidneys but some passing to the oviduct. Knowledge concerning the intrinsic renal innervations is equally sparse. Gilbert (1969) mentioned that relatively few nerves are found within the kidneys, while Bennett and Malmfors (1970) described a few varicose adrenergic fibres in the renal tissue. Although the kidneys are abundantly supplied with sympathetic nerves and a sparse parasympathetic innervation, their exact physiological function is still disputed. Vagal cholinergic and sympathetic adrenergic fibres are known to regulate activity of the kidney by counter regulation.

Renal nerve stimulation has been shown to produce an increase in plasma renin activity (PRA) and a decrease in sodium excretion (Coote *et al.*, 1972). On the other hand renal denervation caused a decrease in renin contents of the kidney (Stella and Zancketti, 1977) and an increase in Na^+, chloride and water excretion (Kamm and Lewinsky 1965; Bello-rouse *et al.*, 1975). After bilateral renal denervation, a change

to low Na^+ diet produced a continuous and progressively negative Na^+ balance (Dibona and Sawin, 1983). The role of autonomic nervous system in the control of renal function is of continuing interest, as indicated in recent literature (Beenwikes et al., 1981; Seely and Levy, 1981). Although the kidney is richly innervated and has been studied for over a century, the origin and function of all the renal nerves is still the subject of controversy.

Previous studies in this laboratory have highlighted the fact that avian kidney metabolic activities are influenced by autonomic nerve fibres. Vagotomy produced an increase in activities of G-6-Pase, GOT and GPT in pigeon kidney (Verma et al., 1984). They concluded that vagal fibres have inhibitory effect on gluconeogenesis, and vagotomy removed this inhibition thereby activating gluconeogenesis. An indirect effect of vagus on kidney metabolic activities could be through its inhibitory action on the release of corticosteroid from adrenocortical cells (Trutnov, 1970). Vagal transection also enhanced the release of glucocorticoids (Pilo et al., 1985). Pilo et al. (1984) studied the effect of vagotomy on kidney functions and reported that the vagal impulse to kidney is essential for the maintenance of blood sugar level.

Administration of acetylcholine inhibited gluconeogenic as well as glycogenolytic activities (Chapter VI). Similarly,

catecholamine administration also produced several metabolic changes in the kidney of pigeons (Chapter VIII). Administration of norepinephrine, the neurotransmitter secreted by sympathetic nerve fibres, produced more pronounced effect on kidney metabolic activities than epinephrine. However, chemical sympathectomy by treating birds with 6-OHDA produced changes in the kidney metabolism akin to what was observed in epinehrine treated pigeons (Chapter VIII). It was suggested that circulating epinehrine, probably released at a higher rate from adrenal medulla, was exerting its influence on the kidney even when adrenergic nerves were rendered inoperative by 6-OHDA treatment (Chapter IX).

The above mentioned studies indicate that acetylcholine and catecholamines have reciprocal effect on the kidney metabolism possibly in the homeostasis of blood sugar level. The parasympathetic and sympathetic nerve fibres thus could influence the metabolic activities in the kidney through acetylcholine or norepinehrine.

The present study deals with histochemical changes in the kidney of vagotomized and chemically sympathectomized pigeons.

MATERIALS AND METHODS

Adult domesticated variety of blue rock pigeons of both sexes, weighing around 250-300 gms were used in the

experiment. These animals were divided into four groups and were acclimated to laboratory conditions for two weeks, caged in groups and fed ad-libitum. The surgery (vagotomy) performed was as follows.

The first group of pigeons were anaesthetized with ether and 5 cms incision on the dorsal side of the cervical region was made. The vagal trunk was seperated from the surrounding tissues and jugular vein and approximate 10 mm section of it was removed. Thereafter the incision was closed by suturing. Second group of animals were sham vagotomised which involved a similar procedure except that the vagal trunk was lifted and left back. Penicillin was administered after surgery (both sham and vagotomized). The sham-operated and vagotomized pigeons were maintained for 48 hrs without food.

Third group of pigeons was injected with 6-hydroxydopamine (6-OHDA) which is a sympatholytic agent. 6-OHDA (Sigma Chemicals) at a dose of 5 mg/0.5 ml/day dissolved in 0.85%saline with 5 mg of ascorbate. The fourth group of birds was injected with the vehicle. Injections were given for 2 days, 24 hours apart and both the groups of birds were kept in starved condition. On the third day, the birds were killed and kidney was quickly removed. For demonstration of alkaline phosphatase, kidney was fixed in cold 10 % neutral formaline overnight. Then the tissue was fixed on a chuck of a

cryostat microtome maintained at-20° C. Frozen sections of 10-12 μ thickness were cut and transferred to incubation media. The sections were incubated for 6 hours in the media prepared according to Burstone (1962), using Fast Blue B as diazonium salt and AS-MX phosphate as the substrate (Sigma Chemical Co., U.S.A.). The media were prepared fresh and filtered before use. At the end of incubation period, the sections were washed throughly in distilled water and mounted in glycerine jelly. For suitable control the sections were incubated in media devoid of substrate.

Total and neutral lipids were demonstrated in frozen sections which were previously fixed in Baker's calcium formol. Sections were washed in distilled water and stained either with sudan black B or fettrot 7 B for total lipids and neutral lipids respectively (Pearse, 1968).

Acetylcholinesterase (AChE) was demonstrated using the method of Karnovsky and Roots (1964) using acetylthiocholine iodide as substrate.

OBSERVATIONS

Alkaline phosphatase in the kidney Vagotomized Pigeons:

Sham operated pigeon kidney showed moderate alkaline phosphatase activity in PCT, ICT and MCT but sparingly

EXPLANATIONS TO FIGURES - CHAPTER X

Figs : 1 - 6.

Histochemical demonstration of Alk Pase in vagotomized and sham operated blue rock pigeon (120 X).

Vagotomized pigeon : Fig.1 Lobule

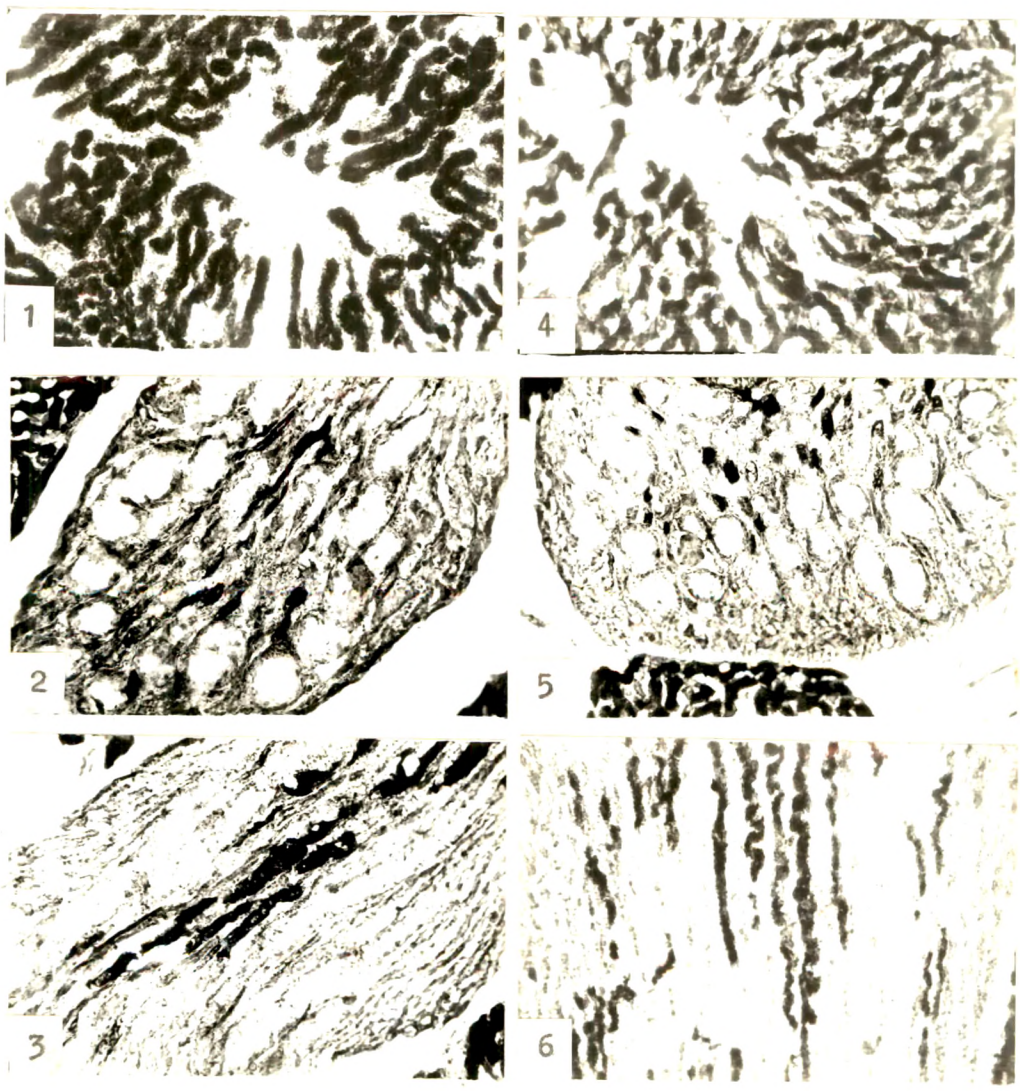
Fig.2 MC

Fig.3 ICT

Sham operated pigeon: Fig.1 4 Lobule

Fig.2 5 MC

Fig.3 6 ICT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EXPLANATIONS TO FIGURES - CHAPTER X

Figs. 7 - 12.

Photomicrographs of the sections of kidney of blue rock pigeon showing histochemical localization of Alk Pase (120 x)

6-OHDA treated pigeons :

Fig. 7 Lobule

Fig. 8 ICT

Fig. 9 MC

Control pigeons :

Fig. 10 Lobule

Fig. 11 ICT

Fig. 12 MC


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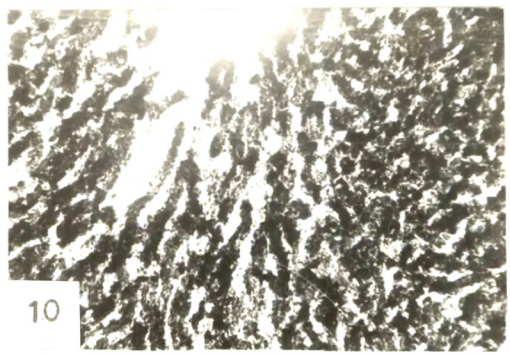
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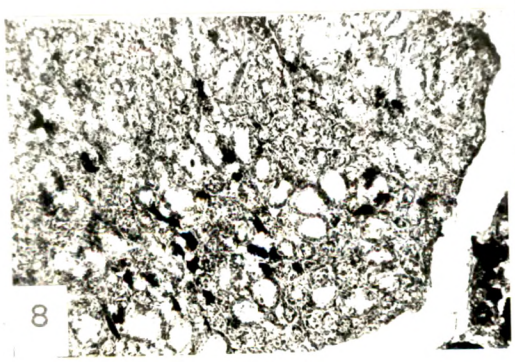
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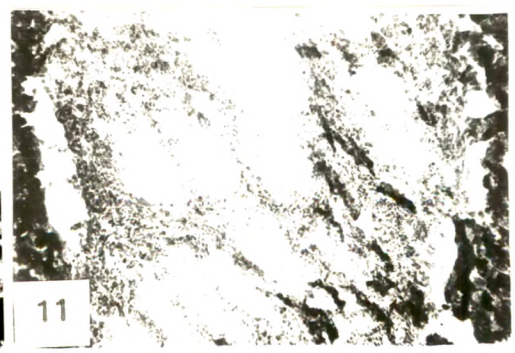
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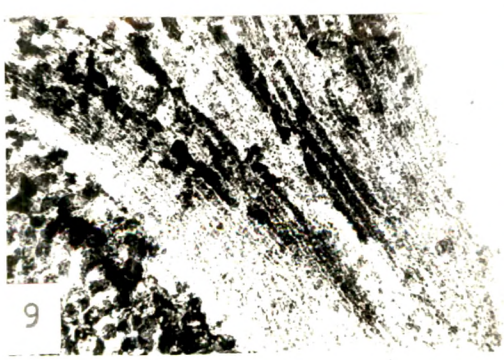
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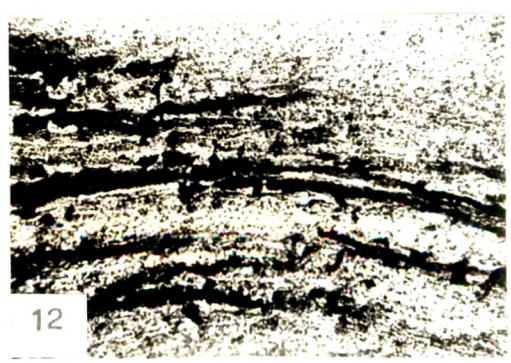
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EXPLANATIONS TO FIGURES - CHAPTER X

Figs : 13 - 16

Photomicrographs of the sections of the kidney of blue rock pigeon showing histochemistry of neutral lipids (120 x)

Vagotomized pigeons :

Fig.13 Perilobular

Fig.14 ICT

Sham operated pigeons :

Fig. 15 Lobule

Fig. 16 ICT

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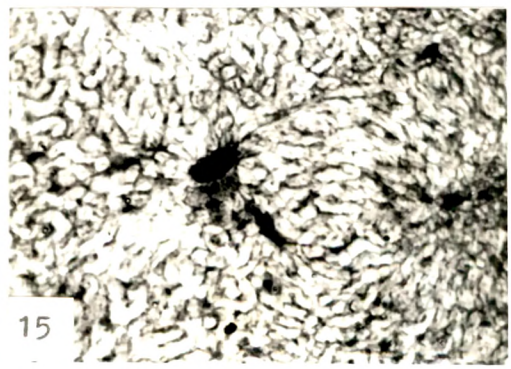

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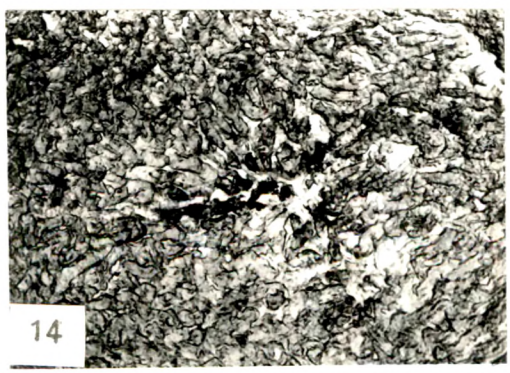

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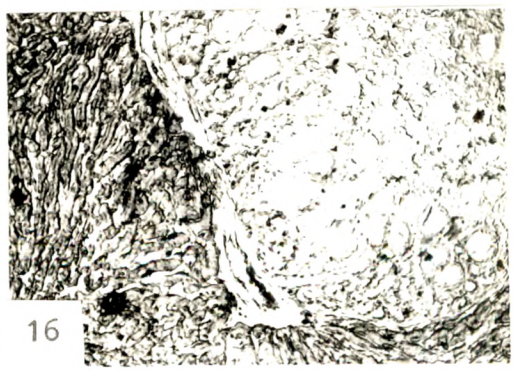

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EXPLANATIONS TO FIGURES - CHAPTER X

Figs. 17 - 22.

Photomicrograph of sections of the kidney of blue rock pigeon showing histochemistry of neutral lipids (120 x)

6-OHDA treated pigeons :

Fig.17 Perilobular region

Fig.18 ICT

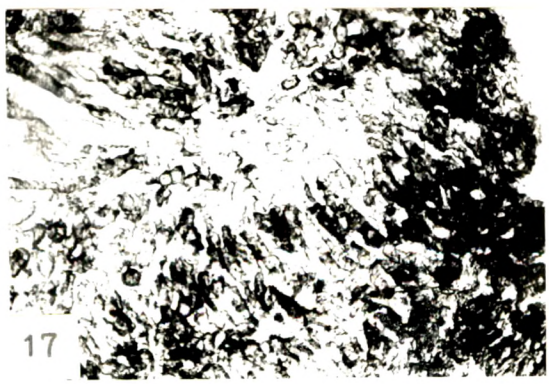
Fig.19 MC

Control pigeons :

Fig. 20 Perilobular region

Fig. 21 ICT

Fig. 22 MC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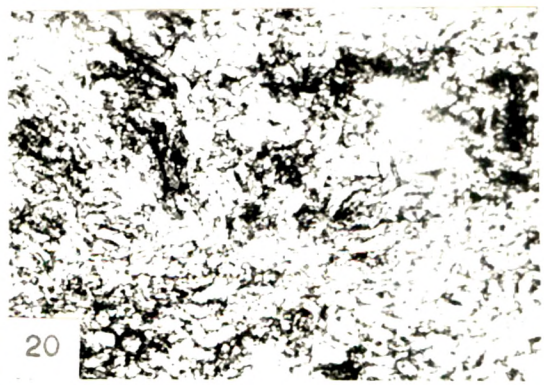
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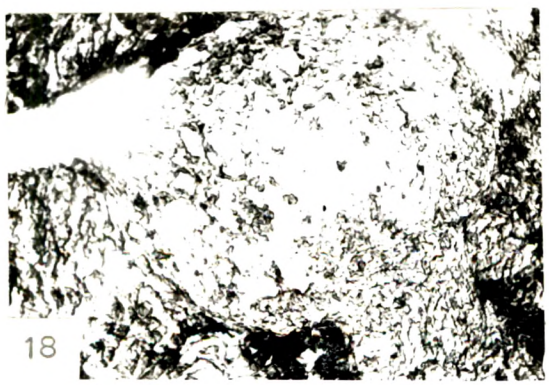
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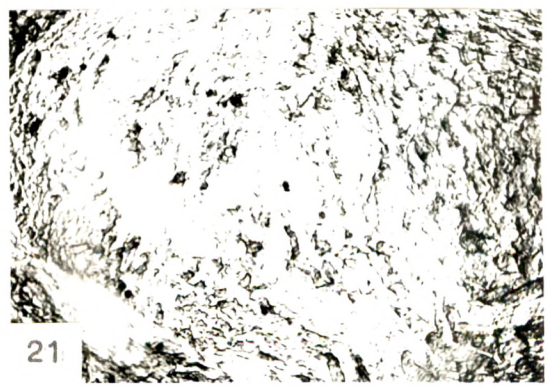
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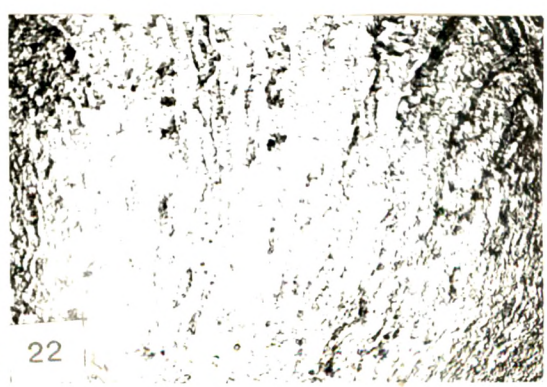
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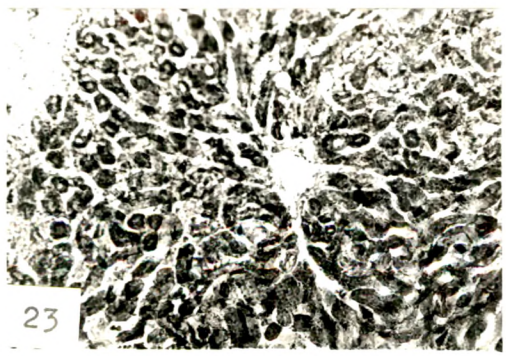
EXPLANATIONS TO FIGURES - CHAPTER X

Photomicrographs of sections of the kidney of blue rock pigeon showing the histochemistry of total lipids (120 x)

Vagotomized pigeon : Fig. 23 Lobule
Fig. 24 ICT
Fig. 25 MT

Sham operated pigeon :
Fig. 26 Lobule
Fig. 27 ICT
Fig. 28 MT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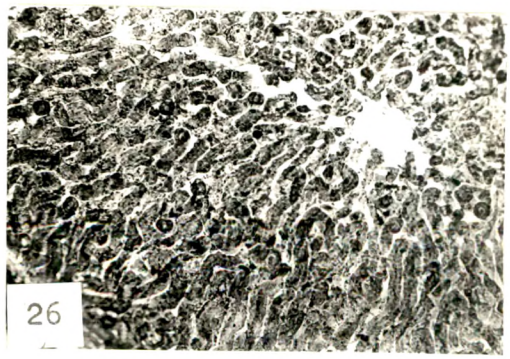
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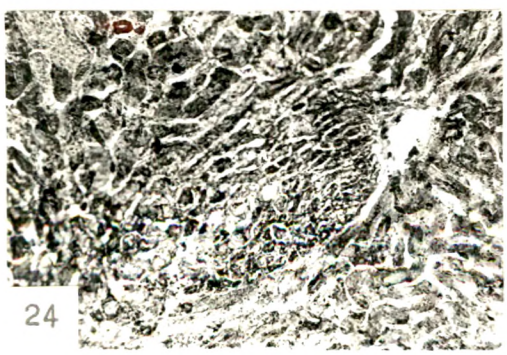
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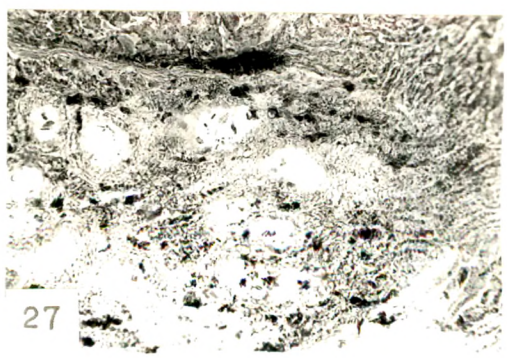
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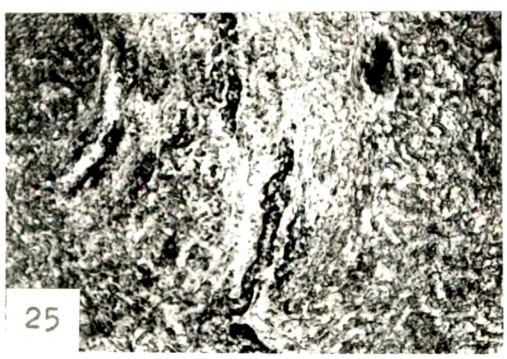
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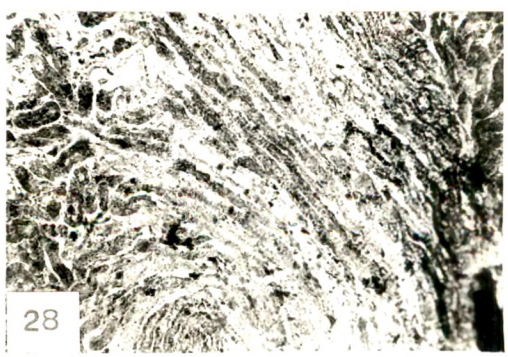
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EXPLANATIONS TO FIGURES - CHAPTER X

Figs. 29 - 32.

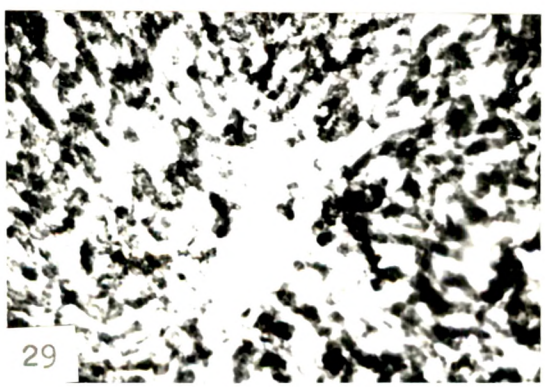
Photomicrographs of sections of the kidney of blue rock pigeon showing histochemistry of total lipids (120 x)

6-OHDA treated pigeon : Fig. 29 Lobule

Fig. 30 ICT

Control pigeons: Fig. 31 Lobule

Fig. 32 ICT



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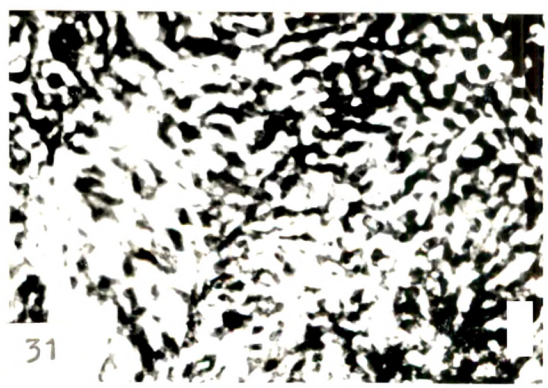

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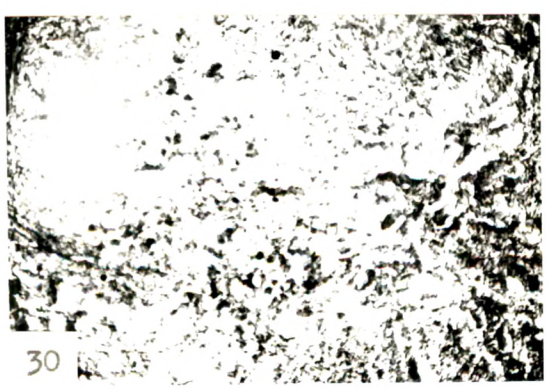

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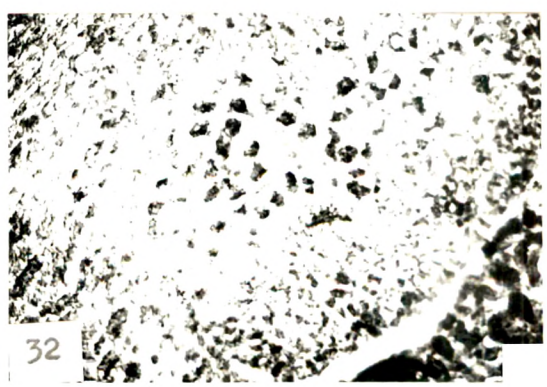

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EXPLANATIONS TO FIGURE - CHAPTER X

Figs. 33 - 38.

Photomicrographs of sections of kidney of blue rock pigeon showing histochemistry of AChE (120 x)

Vagotomized pigeon :

Fig. 33 Lobule

Fig. 34 ICT

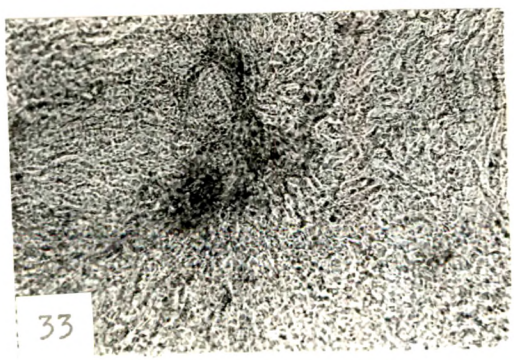
Fig. 35 MC

Sham operated pigeon :

Fig. 36 Lobule

Fig. 37 ICT

Fig. 38 MC



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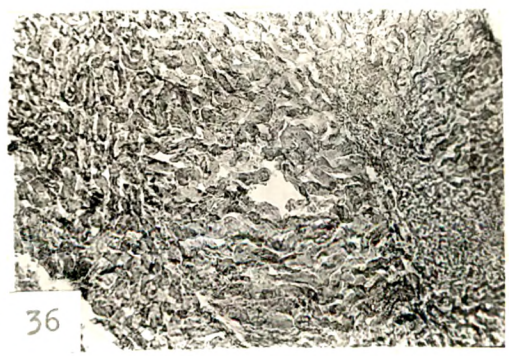

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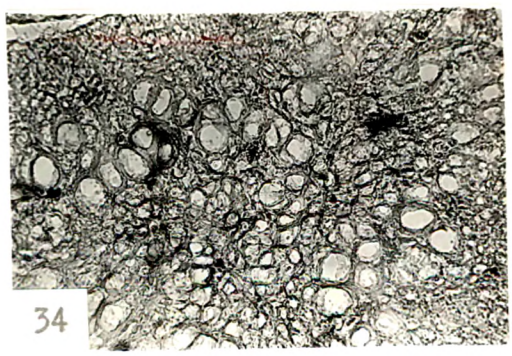

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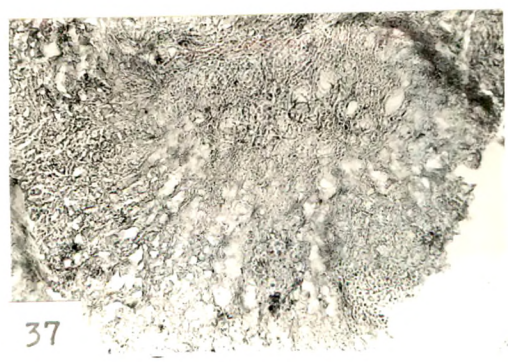

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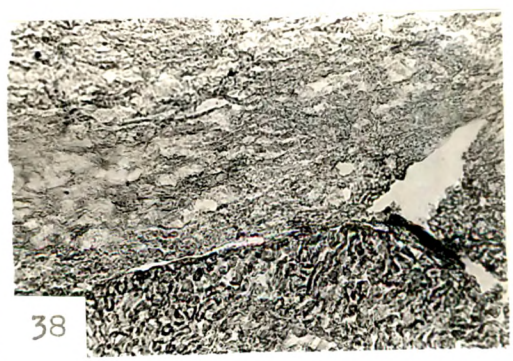

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EXPLANATIONS TO FIGURES - CHAPTER X

Photomicrographs of the sections of the kidney of blue rock pigeon showing histochemistry of AChE (120 x).

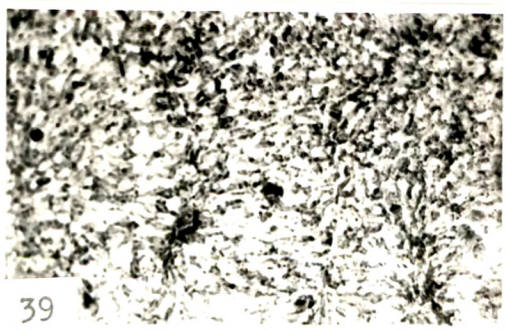
Figs. 39-44.

6-OHDA treated pigeon :

Fig.39 Lobule
Fig.40 ICT
Fig.41 MC

Control pigeon :

Fig. 42 Lobule
Fig. 43 ICT
Fig. 44 MC



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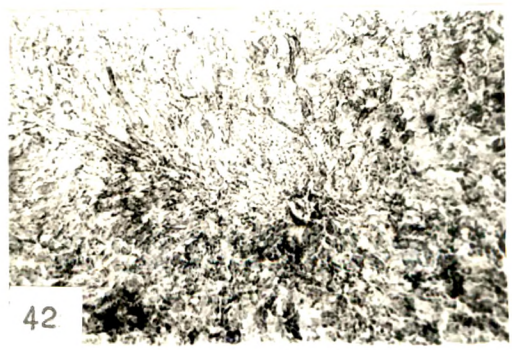

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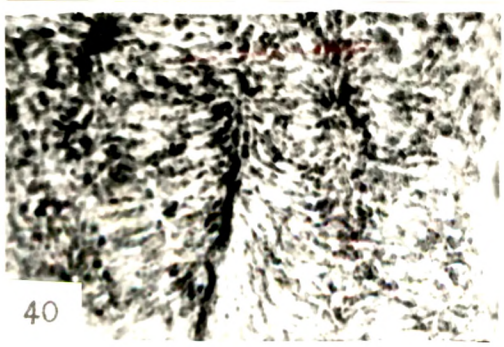

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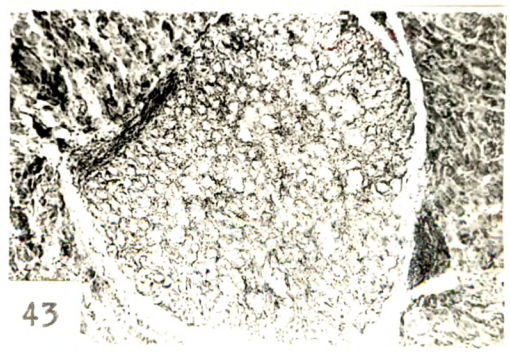

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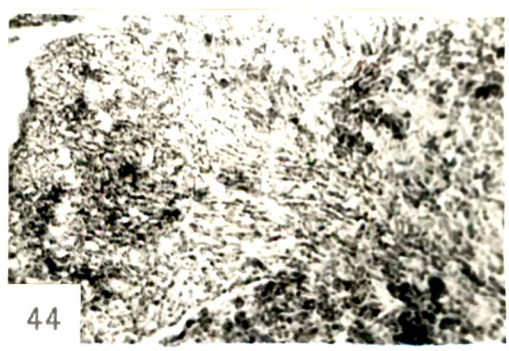

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Table 1: Histochemical reactivities of Alk Pase, AChE and lipids in vagotomized and sham operated pigeon kidney.

	VAGOTOMY					SHAM				
	MC	PCT	ICT	MT	MCT	MC	PCT	ICT	MT	MCT
Lipids F_7	-	+++	+++	++	+++	-	+	+	-	+
Sudan Black	-	+++	+++	++	+++	-	++	+	-	++
Alk Pase	-	+++	+++	+	++	+	++	++	++	+
AChE	+	+	+	+	++	+	++	++	++	++

- = -ve
+ = Low
++ = Moderate
+++ = Intense

MC = Medullary cone
PCT = Perilobular collecting tubule
ICT = Intra collecting tubule
MT = Medullary tubule
MCT = Medullary collecting tubule

Table 2: Histochemical reactivities of Alk Pase, AChE and lipids on 6-OHDA treated and control pigeon kidney.

	6-OHDA					CONTROL				
Enzymes	MC	PCT	ICT	MT	MCT	MC	PCT	ICT	MT	MCT
Lipids F 7	+	++	++	+	++	-	+++	+++	++	+++
Sudan Black	+	++	++	++	++	++	++	++	++	+++
Alk Pase	+++	+++	+++	+++	+++	-	++	++	+++	++
AChE	-	+++	+++	+++	+++	-	+	+	+	++

- = Not detected
+ = Low activity
++ = Moderate activity
+++ = Intense activity

MC = Medullary cone
PCT = Perilobular collecting tubule
ICT = Intra collecting tubule
MT = Medullary tubule
MCT = Medullary collecting tubule

localized in medullary cone (MC) (Figs. 1-3). Vagotomy caused an increase in the histochemical reactivity of alkaline phosphatase in PCT and ICT much more than in other regions (Figs. 4-6).

6-OHDA treated pigeons:

Control birds, showed moderate reactivity of alkaline phosphatase in the PCT, ICT and medullary regions (Figs. 7-9). Chemical sympathectomy caused an over all increase in alkaline phosphatase reactivity in all regions, PCT, ICT, MCT and MC (Figs. 10 to 12).

Lipids in the kidney

Vagotomized pigeons:

Sham operated pigeon kidney showed poor distribution of neutral lipids in PCT, ICT, MCT (Figs. 13 and 14). The total lipids (Sudan Black stained) were also more in cortical regions (PCT, ICT) than in medullary regions (MCT, MC) (Figs. 26-28). Vagotomized pigeons showed increased neutral lipids (Figs. 15 and 16) and total lipids (Figs. 23-25) in all regions.

6-OHDA treated pigeons:

Control pigeon kidney showed a high concentration of neutral lipids (Figs. 20-22) and total lipids (Figs. 31 and 32). When 6-OHDA was injected the kidney showed a reduction

in neutral lipids (Figs. 17-19) as well as total lipids (Figs. 29-30).

AChE in the Kidney

Vagotomized pigeons:

All the areas of kidney in the sham operated pigeons showed only a moderate localization of AChE (Figs. 36-38). Vagotomy caused a decrease in the activity of AChE (Figs.33-35).

6-OHDA treated pigeons:

Control pigeon kidney exhibited a poor localization of AChE (Figs. 42-44) but 6-OHDA treatment produced an intensive reactivity of AChE in all regions (Figs. 39-41).

DISCUSSION

Avian kidney is quite distinct from mammalian one in several respects. The presence of reptilian type (RTN) and mammalian type (MTN) nephrons, renal portal circulation, arrangement of renal tissue into lobes and the arrangement of cortico-medullary regions are all peculiar to avian kidney. Majority of renal tubules are of reptilian type without medullary or nephronal loops, and are confined to cortical region which is the highly vascular region of the kidney. Glomerular filtration rate could be regulated by vasoconstriction. MTN are having nephronal loops and functions just as

mammalian nephrons through a countercurrent gradient mechanism. Salt loading reduces the number of functioning RTNs while MTNs continue to function (Braun and Dantzler, 1972). The cortical regions are highly vascular and are predominantly involved with excretion of metabolic waste materials. This region is also, by and large concerned with metabolic activities. This is evident from the fact that most of the enzymes and lipid deposits are found here. The present study was an attempt to determine whether, neuronal elements have any control over enzyme distribution or functional adjustments between cortical and medullary regions.

Vagotomy increased the lipid deposition in the cortical region, probably indicating some sort of inhibition of metabolic activities in general and lipolysis in particular. On the contrary, chemical sympathectomy reduced the lipid deposition in the cortical region. Vagotomy caused a slight increase in alkaline phosphatase activity in PCT and ICT regions and a reduction in other areas. Quantitatively, vagotomy produced a reduction in alkaline phosphatase activity in the pigeon kidney (Pilo et al., 1984). The histochemical preparations of kidney of vagotomized pigeons showed that alkaline phosphatase activity increased in some areas while decreased in other areas. Similarly quantitative estimations of alkaline phosphatase in the kidney of 6-OHDA treated pigeons showed a decrease in activity, while histochemical localization indicated

an increase. Probably, the different substrates used in quantitative and histochemical studies indicate reactivity of two different groups of enzymes, or differing degree of cross reactivity of enzymes forming the group. Unless further characterization is made, it is difficult to explain the differences in the response shown by alkaline ¡Pase in quantitative and histochemical studies.

Acetylcholinesterase showed a decrease in vagotomized pigeon kidney in the present study while in that of 6-OHDA treated pigeons it showed a 2 fold increase (Chapter IX). In catecholamine administered pigeon kidney also, AChE showed a 3 fold increase (Chapter VIII), although acetylcholine administered pigeon kidney showed no significant change in the activity of AChE (Chapter VI). These observations indicate that AChE activity in kidney increases or decreases in response to activation or inhibition of release of acetylcholine at the cholinergic nerve endings in kidney and not even to external acetylcholine administration. The increase in the activity of AChE seen in chemically sympathectomized or catecholamine administered pigeon kidney could be thus indicative of increased ACh released at the cholinergic nerve endings. This is a counterregulatory response of autonomous nervous system. Similarly, vagotomy caused a decrease in AChE activity mainly due to the fact that ACh release might have ceased altogether after vagal transection.