INTRODUCTION

Although basic anatomy and histology of avian kidney were first studied many years ago by classic descriptive anatomists, the literature of mammalian kidney far surpasses in its volume than those devoted to kidney of this group of vertebrates. Hence, the present understanding of avian kidney, especially its function, often rests upon concepts derived from the study of kidney of mammals. There are several structural differences between avian and mammalian kidneys. The funnel shaped lobular structure, presence of mammalian and reptilian types of renal tubules and the presence of renal portal system provide distinctive features to avian kidney. An excellent comparative account of avian kidney is provided by Johnson(1979).

STRUCTURAL FEATURES OF AVIAN KIDNEY

The paired kidneys are prominent, flattened, retroperitoneal organs on the ventral surface of the synsacrum, and in most birds they are deeply recessed in bony depressions of the synsacrum, termed synsacral fossae. Various morphological and developmental features of the fossae are described by Radu (1975). In the past, anatomists described the avian kidney as consisting of three prominent lobes. Goodchild (1956) correctly pointed out that this was a misapplication of the term "Lobe" and suggested the term "division" as an alternative. The three divisions are termed cranial, middle and caudal renal divisions.

Microscopic anatomy of the avian kidney

The avian cortex is richly supplied with afferent and efferent venous circulation (renal portal and renal veins). Each cortical lobe contains a branch of efferent renal vein called intra lobular (or central) veins (CV). Renal portal vessels (RP) drain into cortical region from perilobular region (Fig.1). Each lobule has a cortical region (CR) and a medullary region (Fig.1). The cortical region is mainly composed of reptilian type of nephrons (RTN) which are devoid of loops of Henle. The mammalian type of nephrons (MTN) with nephronal loops (of Henle) lie in medullary regions. The RTN lie closely packed with their long axis at right angles to the perilobular collecting tubules (PCT) (Fig.3). The large PCTs receive smaller tributaries (ICT) from within the cortical substance.

Medullary region of avian kidney is composed of series of cone-shaped masses. A cone is packed with nephronal loops and medullary collecting tubules (MCT) (Fig.1 and 2). The MCT gradually fuse in dendritic fashion to form a single large collecting duct. These large collecting ducts are also tertiary branches of ureter and several of these ultimately fuse to form a secondary ureteral branch (Fig.2).

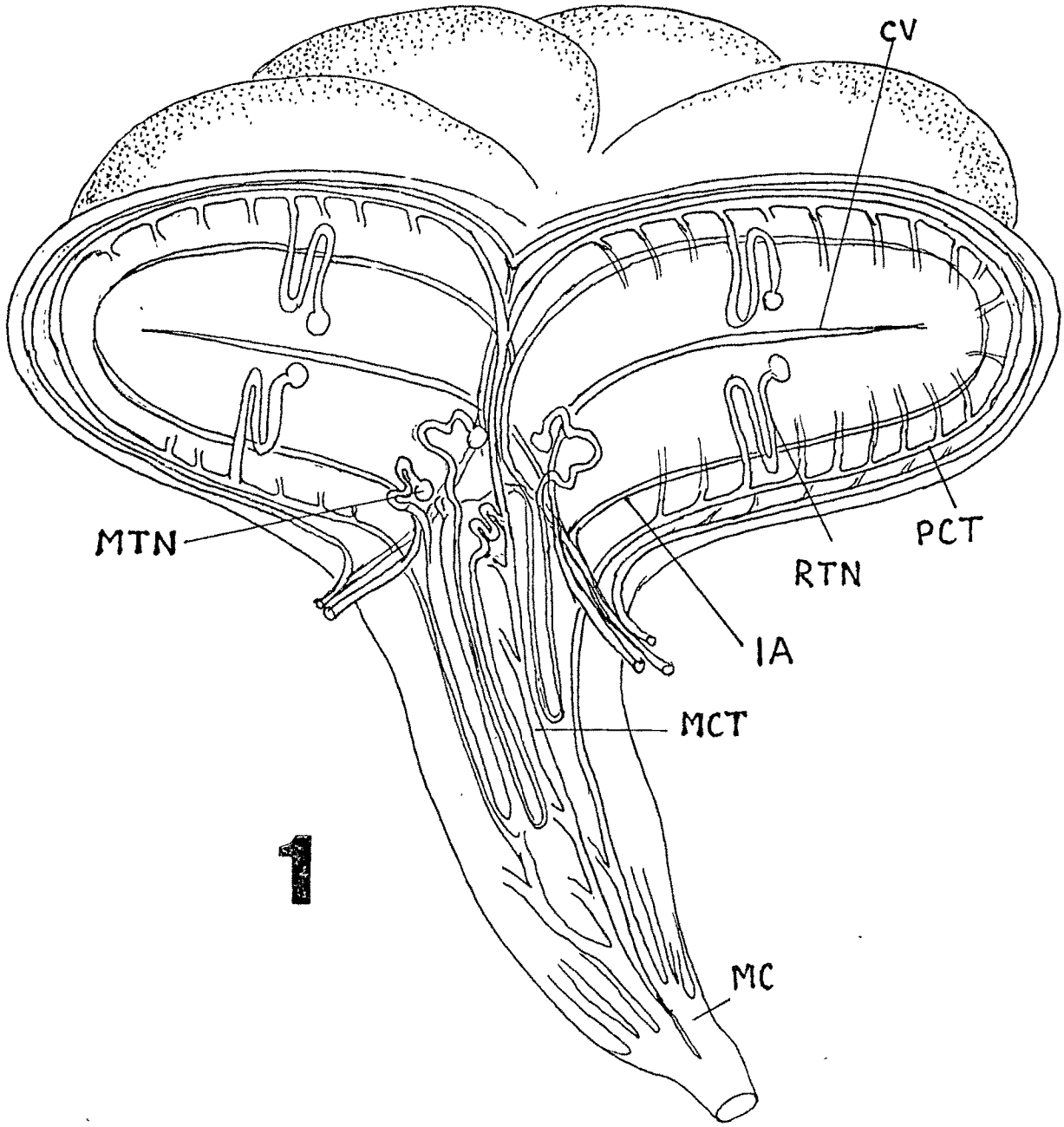
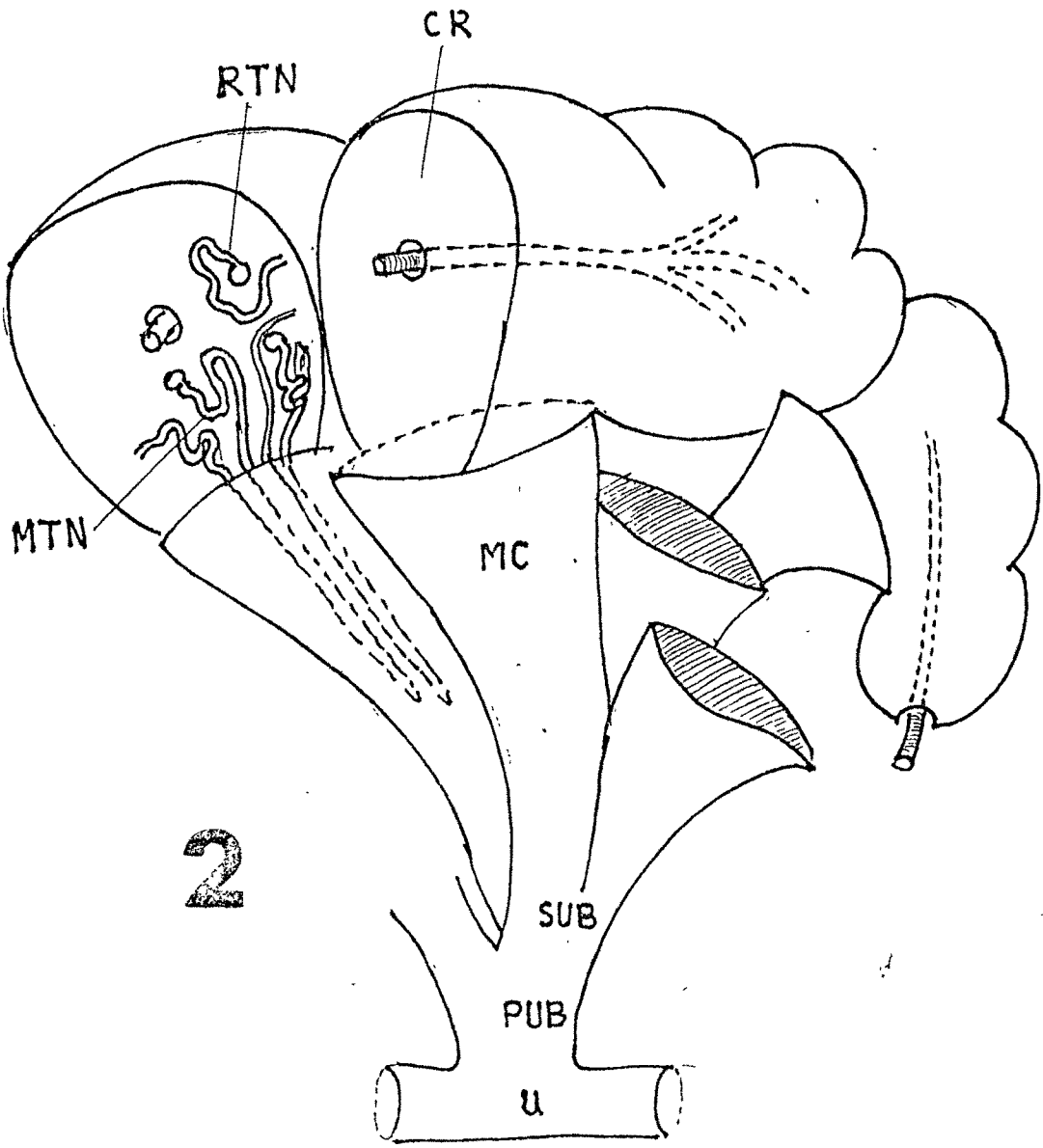


FIG. 1. Diagrammatic representation of one avian renal tubule. CV- efferent intralobular collecting vein; IA- intralobular artery; MTN- mammalian-type nephron; MC- medullary cone; MCT- medullary collecting tubule; PCT- Perilobular collecting tubule; RTN- reptilian-type nephron.

FIG. 2. Diagrammatic representation of cortical (CR) and medullary (MC) regions of avian renal lobules. MTN- mammalian-type nephron; RTN- reptilian-type nephron; PUB- primary branch of ureter; SUB- secondary branches of ureter; U- ureter.



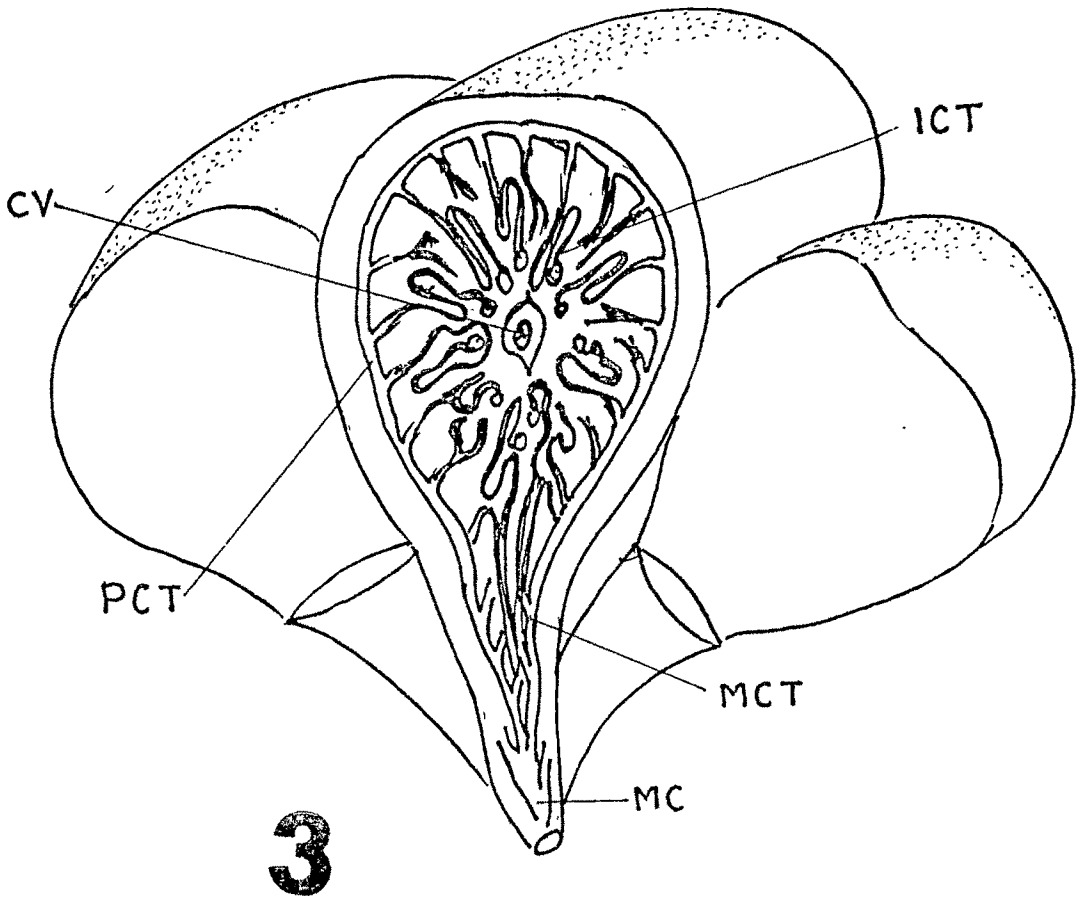


FIG. 3. Diagrammatic cross section of avian renal lobule showing initial collecting tubules (ICT), perilobular collecting tubule (PCT), medullary collecting tubule (MCT) and medullary cone (MC). Efferent intralobular collecting vein (CV) is in the centre of the lobule.

Blood supply and nerve supply:

The presence of a renal portal system in birds is probably the most fundamental difference between avian and mammalian kidney. Blood flow to the kidney is via two systems, arterial and portal. A very extensive peritubular capillary plexus pervades the cortical portion of the lobule (Fig.4). Hence, the reptilian type of nephron and cortical segments of the mammalian type nephrons are literally bathed in blood. Post glomerular arterial blood plus renal portal blood flow together into the capillaries at the peritubular plexus and all subsequent drainage is towards the center of the lobule into an afferent intralobular vein (Fig.4). Several intralobular veins become confluent to form an afferent renal radix; the cranial and caudal renal veins drain into the caudal vena cava.

The nerve supply of the kidney appears to have been very poorly studied. Mauger (1941) described the extrinsic innervation of the kidney coming from the renal plexus, lying at the origin of 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) mentions that relatively few nerves are found within the kidneys, while Bennet and Malmfors (1970) describe a few varicose adrenergic fibres in the renal tissue.

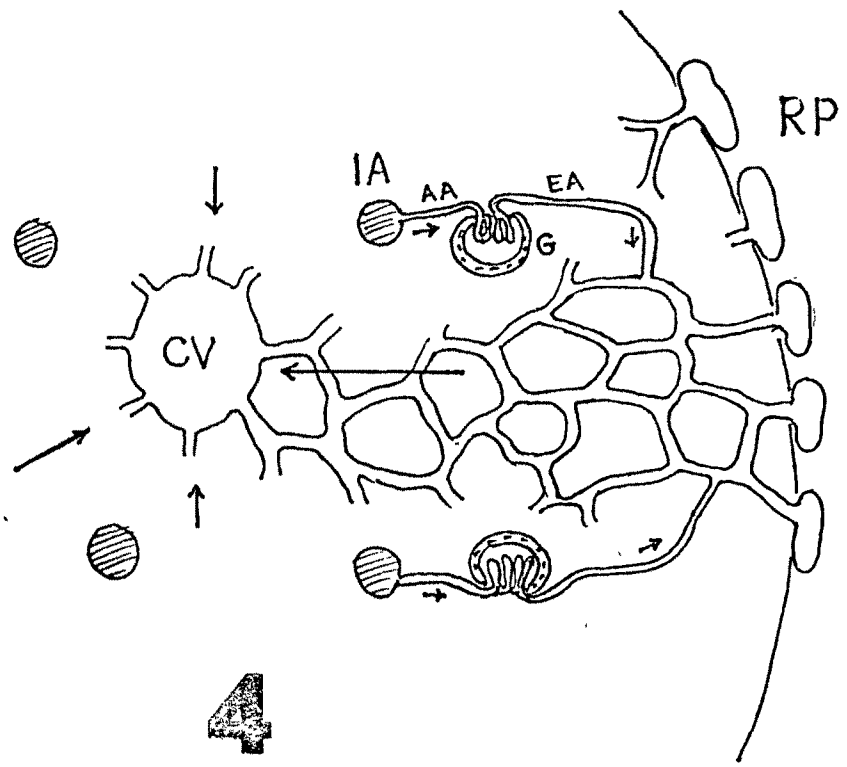


FIG. 4. Diagrammatic representation of intralobular blood supply. AA- afferent artery; CV- intralobular collecting vein; EA- efferent artery; IA- intralobular artery; RP- renal portal vessels; G- glomerulus.

Neural control of renal function:

Kidneys are abundantly supplied with sympathetic and sparse parasympathetic innervation, but their physiological function is still disputed. Vagal cholinergic and sympathetic-adrenergic fibres are known to innervate kidney (Thurau, 1967; Mckenna and Angelakar, 1968; Hashat, 1974; Moffat, 1975) and are also 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 Na^+, chlorine and water excretion (Kemm and Lewinsky, 1965; Bello-rouse et al., 1975). Sinoaortic denervation and cervical vagotomy lead to an increase in sympathetic discharge to the kidney resulting in an increased PRA (Yun et al., 1977). After bilateral renal denervation, changing to low Na^+ diet was associated with continuous and progressively negative Na^+ (Dibona and Sawin, 1983).

The haemodynamic effects of strong renal nerve stimulation and attempted renal denervation have been known for some time. The ability of well functioning denervated kidney transplants to respond appropriately to normal physiologic stresses served to minimize the importance of the renal function. However, several recent observations have served to indicate that pertubations in renal nerve activity within the physiologic range can have major effects on vascular, humoral and tubular responses within the kidney.

A major stimulus for the renewed interest in the renal nerves derives from a broadened prospective of neural anatomy. Studies using 6-OHDA, an agent that selectively destroys adrenergic nerves, indicate that the vessels and tubules receive solely adrenergic nerves (Dibona, 1978). Descrete neurovascular and neurotubular junctions morphologically consistant with sites of synaptic transmission were seen with the electron microscope. These studies provided firm support for a direct effect of adrenergic nerve terminals on renal tubular epithelial cells. Studies of Barajas (1978), in rats, using 6-OHDA, also suggest the distinctive neurovascular and neurotubular junctions.

The physiologic significance of the vascular innervation is well established and the renal nerves are considered to exert an influence on renin secretion (Davis and Freeman,1976). On the other hand, any effect of the renal nerves on sodium reabsorbtion was thought to be secondary to the tubular innervation, by showing an effect of the renal nerves on proximal tubular reabsorbtion of Na^+ apparently independent of any renal vascular changes. Renal sympathetic nerve activity exerts a number of effects that could lead to the development of hypertension. Brody (1978) showed the altered renal sympathetic nerve function with increased catecholamine turnover, and decreased catecholamine content in rats made hypertensive by the administration of de-oxycorticosterone and 1 % NaCl. They suggest that altered renal function in

renal and steroid-salt hypertension primarily results from a reduced capacity of the adrenergic nerves to store norepinephrine.

Metabolic functions of the kidney:

Apart from the excretory and reabsorbtive functions, kidney also plays a role in blood sugar regulation through intense gluconeogenic activity (Krebs, 1963; Exton, 1971; Shen and Mistry, 1979). All the gluconeogenic enzymes are present in the kidney as well as in the liver (Scrutton and Utter, 1968). Kidney slices (Benoy and Elliot, 1973; Lee et al., 1962; Krebs et al., 1963),perfused kidney (Bahlmann et al., 1965; Nishitsutsuji-uwo et al., 1977) and isolated kidney tubules (Guder and Wirthensohn, 1979) were shown to produce glucose using various substrates. Rat kidney was found to synthesize glucose and release it into the blood stream in in vivo conditions (Kida et al., 1978; 1982). Thus, the kidney, although an organ mainly concerned with excretion, also plays an important role in general metabolism particularly so in glucose homeostasis. Kidney may take up compensatory role of producing more glucose when liver functions are altered as in diabetes (Kida et al., 1978).

Gluconeogenesis :

Gluconeogenesis is a vital function which provides mechanism for tissues to produce glucose from non-carbohydrate

precursors. This process comes into operation in starvation or when the diet does not contain carbohydrate. Since the functioning of the central nervous system depends mainly on glucose metabolism, the emergence of gluconeogenesis has gained importance during phylogenesis and ontogenesis. In recent years the role of metabolites and enzymes involved in gluconeogenesis was elucidated. It was also demonstrated that the operation of this pathway is subject to nutritional and hormonal regulatory influences. Krebs (1964) has suggested that in the reversal of the steps involved in the phosphorylation of glucose and fructose-6-phosphate and in the reversal of the conversion of PEP to pyruvate several enzymes are operational which are specific to the gluconeogenic process. The most important function of gluconeogenesis is maintenance of blood glucose level during times when food intake is restricted and/or glycogen stores are depleted. Gluconeogenesis also contributes significantly to the utilization of amino acids, which are either absorbed from the alimentary tract or released during protein breakdown in muscle or other extrahepatic tissues like kidney. Although the liver is the major site of gluconeogenesis in mammals, the kidney may become the major gluconeogenic site during prolonged starvation. In avian system, kidney is the primary site of gluconeogenesis.

Gluconeogenesis in avian kidney:

Gluconeogenesis in avian liver and kidney has received

a lot more attention in recent years. A number of studies with chicken have shown that hepatic glucose synthesis takes place predominantly from lactate (Brady et al., 1978; Watford et al., 1981). Other gluconeogenic substances such as glycerol, alanine, and pyruvate are converted to glucose only at a marginal rate by chicken liver (Brady et al., 1978; Watford et al., 1981). However, chicken kidney could synthesize glucose at an appreciable rate from all precursors (Dickson and Langslow, 1978). The limitation of pigeon liver to convert substrates such as pyruvate to glucose stems from the fact that a cytosolic form of phosphoenol pyruvate carboxykinase (PEPCK) is absent in avian liver which curtails the conversion of oxaloacetate to phosphoenol pyruvate. But avian kidney contains an inducible cytosolic form which greatly facilitates the conversion of alanine, pyruvate and other precursors (via oxaloacetate) to glucose. Hence, in birds, liver converts lactate to glucose while kidney utilizes mainly amino acids to synthesize glucose (Watford et al., 1981; Ogata et al., 1982).

Hormonal control of gluconeogenesis:

The regulation of gluconeogenesis by hormones is a complex process and involves both rapid and long term effects at multiple sites in the gluconeogenic pathway.

Hormonal control of gluconeogenesis occurs at three levels :

1) Involves regulation of substrate supply, which may be long term as well as short term. Much of this important aspect of gluconeogenic control has been done by Scrutton and Utter (1968); Exton et al. (1970) and Exton (1972).

2) The second level deals with a very significant but relatively slow adaptive changes in enzyme activity due to regulation of protein synthesis and/or degradation.

3) The third level is concerned with the minute-to-minute regulation of gluconeogenesis by glucagon, catecholamines, and insulin. These hormones are generally thought to play the most significant role in regulation in the pathway, although adrenal steroids (Exton and Park, 1965), thyroid hormones (Menahan and Wieland, 1967; Singh and Synder, 1978) growth hormone (Tolman et al., 1973; Jefferson et al., 1973) and angiotension II and vasopressin (Hems and Whitton, 1973; Whitton et al., 1978) have all been shown to influence gluconeogenesis.

Glucagon plays a physiological role in the maintenance of the blood glucose through control of gluconeogenesis. Glucagon has a considerable influence over glucose production and gluconeogenesis (Hue et al., 1981). A number of observations in intact animals have suggested that glucagon stimulates gluconeogenesis (Exton and Park, 1968; Feliu et al., 1976). Available evidence do indicate that glucagon exerts regulatory does actions of kidney function in mammals (Baily et al., 1980).

Glucocorticoids are known to have gluconeogenic and diabetogenic actions in mammals (Ingle , 1952; Welt et al., 1952). Long and Lukens (1936) reported that adrenalectomy decreased blood glucose concentration in alloxan diabetic rats. Henning et al. (1966) and Kamin et al. (1967) showed, with kidney slices, that glucose production was decreased by adrenalectomy and increased by administration of glucocorticoids. The rate of gluconeogenic reactions in kidney also increased by administration of glucocorticoids and decreased by adrenalectomy (Flores and Alleyne, 1966; Longshaw and Pogson, 1972). These findings do suggest that gluconeogenesis in renal tissues is controlled by glucocorticoids.

Catecholamines (epinephrine and norepinehrine) stimulate glucose production and simultaneously decrease lactate and pyruvate formation (Blair et al., 1973; Clark, et al., 1974; Kneer et al., 1974; Pilkis et al., 1976; Rogunstad, 1976; Foster and Blair 1978; Kneer et al., 1979; Yip and Lardy, 1981). Most of the actions are α-adrenergic or mediated by NE (Kneer and Lardy, 1983). Studies of Chan and Exton (1978) suggest that E inhibits pyruvate kinase by an α-receptor mediated, c-AMP independent mechanisms by which it stimulates gluconeogenesis. Although there are enough reports on action of catecholamines on gluconeogenesis, experiments in whole animals have yielded conflicting results.

Thyroid hormonal status has significant effects on renal function. In rats surgical thyroidectomy decreased renal plasma flow and glomerular filtration rate, and filtered Na^+ load (Holmes et al., 1970; Katz et al., 1975; Michael et al., 1972). Chu Shek Lo and Theresa (1981) have suggested that thyroid hormones regulates the synthesis of Na^+-K^+-ATPase in the kidney and other target organs at nuclear level. Extensive evidence has been adduced indicating that Na^+-K^+-ATPase is the enzymatic equivalent of the system responsible for active transmembrane Na^+-transport (Lo et al., 1976). T_4 is largely metabolized to T_3 or rT_3 by deiodination in peripheral tissues, which is carried out to varying degrees by all tissues, but kidney and liver are quantitatively important in terms of whole body deiodination because of their size and high T_4-5'-deiodinase activity (Chopra, 1977; Kaplan, 1978). Renal phosphoenolpyruvate carboxykinase shows tremendous changes in hypothyroid and hyperthyroid rats in vivo (Sibrowski et al., 1982). Thyroid hormones are likely to be mediated by cytochrome 'c' reductases activity (Tapbergenov, 1982). They suggested that supraphysiological thyroxine concentration exert a direct action on the enzyme, whereas in physiological levels it regulates cytochrome oxidase, acting as an inducer. Thyroid hormones administration in vivo regulates the activity of hepatic glycogen phosphorylase (Malbon and Campbell, 1982). Kiyoshi and Ichikawa (1982) have reported that there are specific binding receptor for T_3 in rat kidney mitochondrial membrane. Thyroid hormones and

their derivatiges play an important role in the regulation of renal glutaminase (Soakyan and Oganesyan,1982). Thyroid thus has direct effect on number of metabolic reactions. Jolin et al. (1983) showed that thyroidectomized diabetic rats showed only a slight increment in the glucose level in blood, and the treatment with insulin reduced the glycaemic level but not when injected with thyroxine. Thyroxine in this condition produced its gluconeogenic or glycogenolytic activity. Thus it can be seen that thyroxine also helps other hormones in exerting its effects on metabolic reactions.

Neural control of avian kidney gluconeogenesis:

The neural influence on the metabolic activities of the liver is now well understood (Pilo and Patel, 1978; Shimazu, 1983; Lautt, 1983). However, whether such a function could be ascribed to the neural elements in the kidney is much too arbitrary at the present level of understanding. Even in the mammalian kidney no systematic attempt has been done to elucidate the role of nerves in controlling metabolic activies. Most of the studies in mammals are concerned with hypertension, renin release and sodium excretion. In birds even such attempts are fewer. Studies carried out in our laboratory are few exceptions though it was reported that vagotomy caused an increased gluconeogenic activity in the kidney of pigeon (Pilo et al., 1983). It was also suggested that vagotomy may cause a reduction in glucose or sodium reabsorbtion by kidney

tubules (Verma et al., 1984). These preliminary studies have indicated that vagal cholinergic fibres could influence metabolism in the kidney of birds.

Kidney (at least in that of mammals) is only sparsely innervated by parasympathetic fibres while adrenergic fibres are much more prominent and mostly vascular. Adrenergic action in the kidney may be more concerned with regulation of blood flow through kidney and thereby the rate of GFR. However, when the adrenergic nerve endings secrete epinephrine and/or norepinephrine it may be possible that these may also influence metabolic activities in the kidney just as they do in the case of liver.

Aim and Scope of the Present Study:

It is clear from the various studies that are reported so far, that the kidney in general is capable of several metabolic activities. The most significant of all is gluconeogenesis. In mammals, kidney's gluconeogenic capacity is much less than that of liver in normal circumstances. In birds, the rate of renal gluconeogenesis surpasses that of liver. Moreover, avian liver mainly utilizes lactate as a precursor for glucose production while kidney can utilize substrates such as pyruvate, alanine, aspartate and many others.

How many birds so far?

Gluconeogenesis is a metabolic process that has immense importance in the survival of the individual. Hence, this process is under short-term and long-term regulation. Short-term adaptation is required when the individual faces shortage of food, while long-term adaptation is necessary when the food contains little or no carbohydrate. Both short and long-term regulations are mediated by hormones. Since in the liver, short term regulations are also carried out by autonomic nerves, similar role by autonomous nerves could also be expected in kidney.

The nature of actions of hormones and autonomic nerve fibres in the metabolic activities of avian kidney are not clear to date. A series of studies are planned in our laboratory to evaluate whether hormones and nerves could effectively regulate metabolic activities of avian kidney and if they do so in what manner the regulation is exerted at the cellular level. The report presented in this thesis is from the preliminary set of studies.

The chapters presented in this thesis are prepared as seperate entities for clarity of explanation. A certain amount of repetition or overlapping that has crept in was thus unavoidable. In the last chapter, "General Consideration", an attempt is made to interlink the data gathered and to derive an integrated picture.