THE EFFECT OF OBESITY IN THE RAT ON THE KIDNEY AND URINE

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Olaf Mickelsen
Major professor

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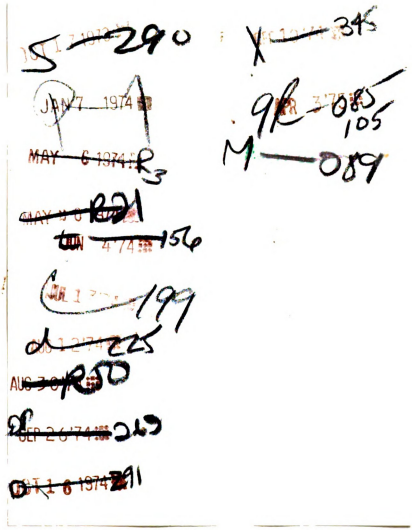

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ABSTRACT

THE EFFECT OF OBESITY IN THE RAT

ON THE KIDNEY AND URINE

By

Jenny Taylor Johnson

Obesity, a major problem in the human population today is associated with an increased incidence of many degenerative and disabling abnormalities including those of the renal-cardiovascular systems. Further investigation was considered necessary to evaluate renal function in the obese state. The purpose of this study was to determine the effect of dietary obesity on renal function and histology in the laboratory rat.

Obesity was produced in Osborne-Mendel male rats by feeding a 60% fat diet with adequate protein, vitamins and minerals.

A light microscopy histopathological examination of kidneys from animals at weaning and at 15, 25, 35 and 45 weeks of age, fed either a control grain ration (GR) or a high fat (HF) ration postweaning, was completed.

The animals fed HF were significantly heavier than the GR controls after 25 weeks of age. These animals contained considerably more fat than the GR animals, especially in the perirenal-retroperitonial depots.

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In the HF-fed group, the right kidney was heavier than the left in animals at 15, 25, 35 and 45 weeks of age. There were no differences in the weights of the two kidneys in the GR groups at any age examined. The total kidney weight was significantly greater in the HF groups at 25, 35 and 45 weeks of age. The kidney weight to body weight ratio was significantly less in HF animals at all ages.

Lesions in kidneys from GR and HF animals affected the glomeruli and tubules. Prior to 35 weeks of age, the incidence of lesions in both groups was almost comparable, although the lesions in the kidneys from HF animals were more severe and covered more of the kidney parenchyma.

By 45 weeks of age and 42 weeks on the respective diets, the kidneys from the HF animals had considerably more damage than those from GR rats. The total number of lesions and the severity of these was greater in the HF group at this age.

The effect of obesity and the HF diet on the accumulation of *p*-aminohippurate (PAH) by renal cortical slices was determined using an *in vitro* slice technique. The accumulation of PAH was significantly depressed in animals fed the HF diet used to produce obesity. Accumulation of PAH decreased with increasing age, body weight and kidney weight independent of diet. Exchanging the diets for a few days or a few weeks affected the transport of PAH, but not of NMN. Animals fed GR for any period of time immediately prior to sacrifice had kidneys which

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The rate of PAH uptake was determined and analyzed kinetically using a Lineweaver-Burk plot. In GR animals the V_{max} and K_m were less in 60 week old animals and K_m values less than the respective age controls. The decrease in apparent affinity and maximal velocity with age and diet could indicate non-competitive inhibition. The differences observed in PAH transport in the HF animals were not the result of differences in oxygen consumption, histology or composition of renal cortical slices. These data are consistent with the presence of some inhibiting factor (as another organic acid) in the serum. However, stimulation not inhibition was demonstrated when serum from the HF or GR animals was added to the incubation medium.

Organic base accumulation was determined in order to demonstrate the specificity of the effect of the HF diet and obesity on organic acid transport. Accumulation of N-methylnicotinamide (NMN) was not different in GR and HF animals. There was no correlation of NMN accumulation with body weight. In HF animals NMN accumulation by renal cortical slices decreased with increased age and kidney weight. Age and kidney weight in the GR animals were not related to NMN accumulation.

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THE EFFECT OF OBESITY IN THE RAT ON THE KIDNEY AND URINE

By

Jenny Taylor Johnson

A THESIS

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Department of Food Science and Human Nutrition

1972

In memory

Mr. a

Mr. a

And in

Mr.

In memory of my grandparents,

Mr. and Mrs. E. C. Taylor

and

Mr. and Mrs. Manning Rogers

And in honor of my parents,

Mr. and Mrs. Woodrow Taylor

This thesis
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Part II.

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INTRODUCTION

Regardless of the criteria used for overweight, the age group studied, or the country, obesity is a common finding. The metabolic and endocrine abnormalities associated with obesity have recently been reviewed extensively (Gordon, 1970; Sims *et al.*, 1971). These include changes in blood lipids, in plasma insulin, glucagon, and adrenal corticosteroids, in adipose tissue and others (Sims *et al.*, 1971). Also associated with obesity are a number of abnormalities in organ function and structure. These include diseases of the liver and cardiovascular system, gynecologic disorders, diabetes mellitus, respiratory disorders and gastrointestinal disturbances.

There have been some suggestions that renal function is impaired in obesity. Most reports, however, are preliminary or limited in scope. Therefore, on the basis of the high incidence of obesity and associated renal-cardiovascular abnormalities in the obese, further investigation was considered appropriate and necessary to evaluate renal function in the obese state. The problems associated with using humans as experimental subjects in an evaluation of the effects of obesity on various physical parameters

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and physiological functions, however, are numerous. Therefore, a number of animal models have been developed.

A variety of chemical compounds when injected into experimental animals can precipitate obesity. These include goldthioglucose (Brecher and Waxler, 1949), monosodium glutamate (Olney, 1969), cortisone (Hausberger and Ramsay, 1953), insulin (MacKay *et al.*, 1940) and bipiperidyl mustard (Rutman *et al.*, 1966). There is no unanimity as to the mechanism(s) whereby the obesity is produced. Despite their limitations, induction of obesity by chemical compounds appears to have less problems associated with it than when obesity is produced with electrolytic lesions.

Obesity resulting from bilaterally lesioning the hypothalamus in rats without hypophyseal involvement was first reported by Hetherington and Ranson (1939). Electrolytic destruction or electrocauterization by radio frequency current of the ventromedial nuclei of the hypothalamus in a number of species may produce obesity. Several disadvantages exist when hypothalamic obesity is used as an experimental model. These may include changes in organ function, metabolism, and growth. Hypothalamic obesity is a useful model in investigating the neural factors that may regulate food intake of the organism.

Parasitic obesity has been produced in mice, rats, deer mice and hamsters. Increased weight gains and growth occurred in mice, for example, after the injection of

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spargana of *Spirometra mansonoides* (Mueller, 1963). The mechanism involved in this parasitic-induced weight gain has not been determined.

A number of experimental models of genetic obesity exist. These have recently been extensively reviewed by Bray and York (1971). They include the "yellow" obese mouse, the adipose mouse, the *ob/ob* or AO mouse, the NZO mouse and the "fatty" rat. These models can be useful in an attempt to understand the metabolic and biochemical varieties of human obesity.

Researchers have developed a number of other procedures which do not subject animals to operative procedures or injections of chemicals and are perhaps most comparable to the obesity in humans. These include force feeding, feeding high caloric rations, restricting activity or a combination of these (Fenton *et al.*, 1951; Ingle, 1949; Cohn, 1961, 1963; Mickelsen *et al.*, 1955). The Osborne-Mendel rat has a propensity for obesity when fed a 60% fat diet. This model had previously been used extensively in the laboratory in which my research was conducted (Schemmel *et al.*, 1969).

On the basis of the high incidence of obesity and associated renal-cardiovascular abnormalities in obese humans and of previous laboratory studies of kidney function in the obese animal, further investigation was appropriate and necessary to evaluate renal function in the dietary obese rat. The kidney function tests were selected on the

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basis of their sensitivity, their applicability to this study, their usefulness in evaluating various parameters of kidney function and the resources available. The experiments were designed to test the hypothesis that dietary obesity adversely affects kidney function in the laboratory rat.

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PART I. Review of literature.

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The Functions of the Kidney

The physiological and biochemical functions of the kidney must be understood prior to selecting tests of kidney function and assessing their results. The kidneys hold the key position in controlling the homeostasis of the internal environment. They accomplish this (1) by removing waste materials and substances that are useless to the body, regardless of their concentration in the blood and whether they are end products of metabolism (as urea, creatinine, etc.) or are exogenous substances (drug metabolites, phenol red, heavy metals, etc.); (2) by participating in the regulation of the acid-base balance of body fluids; (3) by regulating electrolyte composition and osmotic balance; (4) through the production and release of renin and aldosterone; (5) through the production and release of erythropoietin; and (6) by the conservation and reabsorption of substances valuable to the body economy, such as glucose, protein, amino acids, etc. when their concentration is commensurate with bodily demands and their quantity does not exceed the maximal capacity of the renal tubular mass. The kidney is second only to the liver in synthetic activities and in other biochemical functions of the body. The kidney may also serve as a storage organ. Okuda (1962) has shown that the rat kidney stores vitamin B_{12} that has been absorbed

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in excess of the body requirement and releases it when the need arises. The kidney is the site of the conversion of vitamin D_2 or vitamin D_3 to the active metabolite, 1,25-dihydroxycholecalciferol (Gray *et al.*, 1971).

The kidney is an active site of glucose formation. In addition it can synthesize amino acids, protein, mucoproteins and fats.

The role of the kidney in protein metabolism has been recently reviewed by Cahill and Owen (1970). Since the kidney is the primary excretory organ for nitrogen, the kidney's role in overall nitrogen homeostasis is an important one.

Urea excretion is primarily a function of the blood urea concentration, so the kidney's role is passive. Ammonia excretion, in contrast, is actively regulated by the kidney. Nash and Benedict (1921) demonstrated that renal tubular cells form ammonia from precursors in arterial blood and secrete it in high concentration into tubular urine. Glutamine is the primary precursor; α-amino nitrogen of other amino acids may also be used in ammonia formation.

The kidney may also serve as a proteolytic organ, but probably only to small protein or peptide molecules capable of passing through the normal glomerulus. Under abnormal conditions (proteinuria, nephrotic syndrome, etc.) which led to an excessive accumulation of protein molecules of small molecular weight, or where there is

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increased glomerular permeability to large molecules, the kidney may be a major site for protein catabolism (Cahill and Owen, 1970).

In contrast to other organs such as the liver, heart and muscle where many endogenous substrates are utilized, the kidney utilizes relatively few substrates for its energy requirements *in vivo* (Cohen, 1964). The major endogenous renal substrates are found in the plasma and include nonesterified fatty acids (especially palmitate), lactate, glutamine and α-ketoglutarate.

The nephron is the functional unit of the kidney. The processes it uses to accomplish its job are filtration, reabsorption and secretion.

The kidneys receive approximately one-fourth of the cardiac output. From this volume, the kidneys remove by filtration a portion of the blood with dissolved products, excesses of electrolytes, and foreign materials.

About 99% of the material filtered at the glomerulus is returned to the blood through the reabsorption process as the filtrate passes through the nephron. Substances reabsorbed in the proximal and distal tubules and in the loop of Henle include water, sodium, glucose, amino acids, bicarbonate and vitamins.

Secretion is the process by which proximal and distal tubular cells transport materials from peritubular fluid to the tubular urine. Substances secreted include ions such as NH_4^+ and K^+ and foreign substances such as penicillin, phenol red, and bromcresol green.

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The Effect of Diet on Kidney Function, Size and Composition

According to Kark (1968), disturbances of kidney functions and structures in animals have been produced with some difficulty by dietary manipulation. However, nutrition as a factor influencing kidney structure and function in humans and animals has often been underestimated. Kidney composition, size and function may be affected by dietary deficiencies, excesses or imbalances. The following is a review of several dietary treatments which may affect the kidney in humans and animals.

Malnutrition

Humans:

The effect of malnutrition (various states of starvation or protein-calorie malnutrition) on kidney function in humans depends in part upon the age of the individual when the dietary restriction is imposed. Klahr *et al.* (1967) states that "careful and complete studies of renal function during severe malnutrition are scarce." An examination of the literature substantiated his claim.

The question of whether malnutrition affects kidney function in the adult has not been sufficiently determined. Studies have been limited primarily to observations of persons in prison or detention camps during or after wars. Earlier reports of the effect of starvation on kidney

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function were reviewed by Keys *et al.* (1950) and McCance (1951). Only a few of the starvation studies after World War II contain data on kidney function. Furthermore, these data are incomplete, with emphasis on tests of concentration and dilution. Despite the scarcity of recent data, however, several conclusions can be made.

The most commonly described renal disturbances in malnourished patients are polyuria and nocturia (Mollison, 1946; Keys *et al.*, 1950; McCance, 1951; Klahr *et al.*, 1967). These symptoms were described in detail in World War I victims by Schittenhelm and Schlecht in 1918 according to Klahr *et al.* (1967).

Semistarved individuals have an increased urine volume (Keys *et al.*, 1950; McCance, 1951; Alleyne, 1966; Klahr *et al.*, 1967). For example, the volume of urine from semistarved subjects in the Minnesota study was 3-4 liters in 24 hours (Keys *et al.*, 1950). Mollison (1946) reported no abnormalities in examination of urine from starvation cases at the Belsen detention camp. However, determinations of glomerular filtration rate (GFR) and renal plasma flow (RPF) were made on only 4 subjects and results were inconclusive. McCance (1951) reported that undernourished men in Wuppertal responded to a water dilution test normally, but were unable to compensate when water was withheld. Edema, not abnormal kidney function, was responsible for this, according to McCance (1951). Subjects in the Minnesota starvation study had

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polyuria (Keys *et al.*, 1950); however, no specific determinations of renal function in these men were actually made.

Renal function was depressed in obese persons starved for weight reduction as evidenced by 50% decreases in GFR and C_{PAH} (Edgren and Wester, 1970).

In a carefully controlled experiment of protein malnutrition in 11 adults, Klahr *et al.* (1967) reported that a defect in renal concentrating ability was present. Urine osmolality following 14 hours of fluid deprivation never exceeded 600 mOsm per kg H_2O in the malnourished state; whereas, in normal subjects under similar conditions this value approached 800 mOsm per kg H_2O (Klahr *et al.*, 1967). This concentrating defect was reversible after 36-141 days of protein repletion.

The plasma potassium concentration was normal in subjects in the malnourished state; therefore, Klahr *et al.* (1967) reasoned that the impairment of the urinary concentrating mechanism was not a result of a concomitant potassium depletion. Decreased urea concentration in the renal medulla was suggested as the most likely cause of the concentrating defect in malnourished patients (Klahr *et al.*, 1967).

Srikantia (1968) reported that renal clearances of inulin and diodrone in children suffering from kwashiorkor and in adults with nutritional edema were not

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abnormally low. He suggested that antidiuretic hormone (ADH) plays a causative role in the genesis of the edema of protein-calorie malnutrition (Srikantia, 1968).

Some altered kidney functions and histologic degeneration occurred when young humans were malnourished. Glomerular hyalinization and pericapsular fibrosis were seen in kidneys from infants and young children dying from kwashiorkor (Davies, 1948). Stirling (1962) reported that 22 of 31 Jamaican children suffering from malnutrition had renal lesions. Furthermore, he suggested that there may be residual renal damage even if the infants recover from the malnutrition (Stirling, 1962).

In contrast to Stirling (1962), Alleyne (1966) found that kidney function in children improved with recovery. Prior to recovery, malnourished children had depressed glomerular filtration rates (GFR) and renal plasma flow (RPF) values. Furthermore, such indicators of impaired tubular function as amino aciduria, renal phosphaturia, impaired urinary concentrating ability and an inability to excrete an acid load in these children improved on refeeding (Alleyne, 1966).

Malnutrition in a number of forms appears to influence various kidney functions in children and adults. Further carefully controlled studies investigating a number of different kidney functions are required.

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Animals:

A number of animal studies suggest that renal size and function are affected by various states of malnutrition. Dicker *et al.* (1946) placed rats on turnip, carrot and low casein diets that were markedly deficient in protein and calories. After 36 days on the diet, the rats did not respond normally to water concentration and water dilution tests. Concentration tests showed a large loss of the ability to produce a concentrated urine; the maximum specific gravity dropped from 1.070 in the control period to 1.041 after 36 days of dietary restriction (Dicker *et al.*, 1946). However, this observation could be related to the possible retention of some of the administered water as edema fluid in the malnourished animals and may not reflect dietary damage to the kidney *per se*.

Furthermore, glomerular filtration rate increased in the semistarved state as the urine flow rate increased (Dicker *et al.*, 1946). This suggests that the semistarved rat produces a diuresis by increasing the volume of glomerular filtrate rather than by decreasing tubular reabsorption as does the normal rat. Renal plasma flow also increased in the rats on the deficient diets in proportion to the rate of urine flow. The total tubular excretory mass (T_m) of diodone in the malnourished animals was about 30% of the control value (Dicker *et al.*,

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1946). In addition, Krebs *et al.* (1963) reported that renal gluconeogenesis increased in rats starved for only 48 hours.

Much work has recently been centered on the effect of maternal malnutrition on the kidneys of the offspring. A reduction in relative kidney size (as a percent of body weight) was reported for newborn rats delivered by dams fed a protein restricted ration (Zeman, 1967). This is important since several workers have proposed that organ size is related to the degree of maturation (Potter and Thierstein, 1943). Furthermore, total RNA and DNA were decreased in these kidneys at 20 days of gestation (Zeman and Stanbrough, 1969). Animals nursed by dams subjected to protein deficiency during gestation also had lower kidney weights than controls (Zeman, 1970). Furthermore these kidneys had less RNA, DNA and total protein (Zeman, 1970).

Alkaline phosphatase, nonspecific esterase, leucine-aminopeptidase, ATPase, and acid phosphatase were studied histochemically in kidneys from newborn rats (Zeman, 1968). Alkaline phosphatase has long been considered a sensitive indicator of the functional capacity of the kidney (Brain and Kay, 1927). It is suggested that this

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enzyme, which is located in the brush border of the proximal convoluted tubules in the rat kidney (Wachstein, 1955) presumably functions in tubular absorption phenomena (Wilmer, 1944). The acid phosphatase is used as an indicator of the presence of lysosomes (Straus, 1954; Miller and Palade, 1964). Zeman (1968) found no change in ATPase, nonspecific esterase or leucineamino-peptidase activity in kidneys from control and maternally protein restricted rats. However, levels of alkaline and acid phosphatase were decreased (Zeman, 1968), indicative of retarded development since enzymogenesis is considered to be an aspect of differentiation according to several workers (Moog, 1952; Verne and Hebert, 1964).

Renal immaturity was further suggested in the protein-deficient young as indicated by increased quantities of mesenchymal-like connective tissue, fewer identifiable glomeruli, a larger proportion of immature glomeruli and decreased proximal tubules or shorter tubules (Zeman, 1968).

In a follow-up study Hall and Zeman (1968) measured kidney function in newborn, 2-, 4-, and 6-day old rats using water diuresis, osmotic diuresis, and inulin clearance. The clearance of inulin in control animals was four times that of the protein-deprived rats. Urine

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excretion was decreased in the experimental animals in water and osmotic diuresis tests (Hall and Zeman, 1968).

Chow and co-workers investigated kidney function in offspring from dams fed, during gestation and lactation, a normal ration restricted to 50% of that consumed *ad libitum* by controls (Lee and Chow, 1965, 1968; Roeder and Chow, 1969). Animals from these underfed dams, although fed *ad libitum* post weaning, excreted greater amounts of nitrogen. Furthermore, there was an abnormal distribution among the nitrogen-containing components of the urine (Lee and Chow, 1965). Animals from restricted mothers had a lower mean nitrogen retention at 6, 10, 14 and 18 months. Urine from rats born to dams fed restricted amounts of the ration during pregnancy and lactation had a significantly larger proportion of total amino acids and a significantly smaller proportion of urea, creatinine, and ammonia (Lee and Chow, 1965). This increased urinary excretion of amino acids could result from high blood levels of these, or impaired renal tubular absorption.

Urine from experimental animals at 3 months of age had a larger percent of ammonia than that from control animals. This suggested to these workers that in the animals from restricted dams there was an impaired acid-base balance or a higher level of circulating amino acids as urinary ammonia comes from hydrolysis of blood glutamine in the kidneys and oxidative deamination of

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blood amino acids. In later work Lee and Chow (1968) showed that progeny from restricted animals excreted more free basic amino acids, such as arginine and histidine.

Recently, Roeder and Chow (1969) looked at the concentrations of RNA, DNA and activities of glutaminase I and cathepsin in the kidneys from progeny of restricted animals. At 13 months of age there was no significant differences in these parameters. At 19 months, glutaminase I activity per unit wet weight was the same in progeny from dams restricted during gestation and lactation and control groups although both groups were fed *ad libitum* post weaning. When this activity was expressed per unit of DNA to estimate enzymatic activity per cell, the values were lower in the animals from restricted dams. Cathepsin activity per unit wet weight or per unit of DNA was higher in progeny from restricted dams than in control groups. It increased significantly in the restricted animals between 13 and 19 months of age. These data suggested the early appearance of senescent changes in the kidneys from progeny of dams restricted in feed consumption during pregnancy and lactation (Roeder and Chow, 1969).

Widdowson and McCance (1956) could detect no differences in loss of kidney weight as a proportion of body weight in growing and adult rats subjected to complete starvation and to undernutrition. Stewart (1919) reported that kidney weights were increased in rats subjected to

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repeated periods of fasting from birth until age 11-22 days. In contrast, Brown and Guthrie (1968) found the absolute weight of kidneys in rats deprived during and after weaning was less than that in control animals. Furthermore, upon refeeding these animals a nutritionally adequate diet for up to 10 weeks, kidney weights were less than 80% of those of control rats (Brown and Guthrie, 1968).

As reviewed above, there is considerable evidence that malnutrition affects renal function in animals.

Potassium

Humans:

Renal abnormalities are produced in humans by a potassium deficiency. According to Conn and Johnson (1956), kaliopenic nephropathy should be recognized as an established clinical and pathologic entity.

Conn and Johnson (1956) reviewed the early reports of renal tubular lesions accompanying cases of chronic dysentery, bacillary dysentery and other chronic intestinal diseases. Perkins *et al.* (1950) were the first to associate these renal tubular changes with a potassium deficiency. Since then several reports have related the tubular lesions ("clear cell nephrosis" or "vacuolar nephropathy") to the state of potassium deficiency associated with chronic intestinal disease (Keye, 1952; Schwartz and Relman, 1953; Achor and Smith, 1955).

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A hypokalemic state resulting from excessive use of laxatives produced impairment of renal function in two humans studied by Schwartz and Relman (1953). These subjects had a depressed clearance of *p*-aminohippurate and an inability to concentrate urine maximally (Schwartz and Relman, 1953).

Humans with primary aldosteronism show the same tubular lesion morphologically that have been observed in autopsy cases with histories of potassium depletion resulting from some chronic intestinal disease (Conn and Johnson, 1956). Furthermore, these clinical cases of primary aldosteronism show histologic lesions similar to those in animals on a potassium-deficient diet (Schrader *et al.*, 1937; Follis *et al.*, 1942; Follis, 1943; Newberne, 1964; Segar and Schulz, 1965) or fed an adequate diet but given large doses of desoxycorticosterone (Darrow and Miller, 1942). Muehrcke and Rosen (1964), however, suggested that the site of alterations in the kidney may not be the same in potassium-deficient humans, as in potassium-deficient animals. Whether potassium deficiency produces changes in urinary diluting capacity is unclear (Levitin *et al.*, 1960).

Renal lesions resulting from an induced depletion of potassium in adult humans appear to be reversible (Blahd and Bassett, 1953; Fourman, 1954; Evans *et al.*, 1954; Womersley and Darragh, 1955; Clarke *et al.*,1955).

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Animals:

As a result of many studies in animals, it is evident that renal cells, especially tubular cells, need adequate potassium to preserve their integrity. In fact, Hollander *et al.* (1958) suggested that permanent renal damage may result from an acute episode of potassium depletion. They observed that rats after 3 days of potassium deficiency followed by adequate dietary potassium were unable to concentrate urine maximally.

In contrast, Holliday and Egan (1962) found that rats on a potassium deficient diet for 30 days had GFR and C_{H_2O} values which were less than those in controls. When these animals were refed potassium for 5-13 days, values for GFR and C_{H_2O} returned to normal (Holliday and Egan, 1962). Segar and Schulz (1965), exposed rats to repeated periods of potassium deficiency interspersed with periods of adequate dietary potassium. They reported that although kidneys from these rats showed tubular dilation, the rats could concentrate urine maximally.

Microscopic tubular lesions in rats, mice, dogs, cats and monkeys with potassium deficiency have been reported (Schrader *et al.*, 1937; Follis *et al.*, 1942; Follis, 1943; Newberne, 1964; Muehrcke and Rosen, 1964; Segar and Schulz, 1965).

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Concentrations of the enzymes, glutaminase and carbonic anhydrase are increased in kidneys from potassium-deficient rats (Iacobellis *et al.*, 1954). Polydipsia and polyuria (Brokaw, 1953), decreased creatinine clearance (Muntwyler and Griffin, 1953), renal hypertension (Grollman and White, 1958), increased protein excretion (Morrison and Gardner, 1963), and renal hypertrophy (Durlacher *et al.*, 1942; Follis *et al.*, 1942; Fuhrman and Brokaw, 1951; Brokaw, 1953; Muntwyler and Griffin, 1953; Perey *et al.*, 1967a, 1967b) also occurred in potassium-deficient animals.

Inclusion of rubidium and, to a lesser extent, cesium in potassium-deficient diets protected rats from the renal lesions normally seen with potassium deficiency (Follis, 1943). The distribution, biological properties and excretion of rubidium are very similar to those of potassium. Rubidium was actively transported into the rabbit lens in competition with potassium (Becker, 1962). Furthermore rubidium replaced potassium in the activation of Na-K ATPase in this system (Bonting *et al.*, 1963). Whether rubidium acts similarly in the kidney has not been determined.

Perey and co-workers (1967a, 1967b) induced multiple cysts in the kidneys of fetal rabbits. A single injection of a long-acting adrenal corticosteroid (as 9-fluoro-prednisolone) into the rabbits at birth produced cystic changes in the kidneys. These changes could be prevented

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by repeated injections of potassium chloride (Perey *et al.*, 1967a, 1967b). Therefore, the hypokalemia or a general systemic effect produced by the corticosteroids could be the causative factor (Perey *et al.*, 1967a, 1967b). This finding could have great implications considering the problem of congenital polycystic renal disease in man.

Additional evidence for the kidney's requirement for potassium comes from tissue culture studies. Crocker and Vernier (1970) found that the fetal mouse kidney required a high concentration of potassium for normal development.

Sodium

Humans:

Information concerning various states of sodium status and renal histology and function in humans is sparse.

Black *et al.* (1950) reported altered tubular function in humans fed a salt-poor diet for only a few days. The reabsorption of sodium by the renal tubules was increased 1-2 hours after hypertonic saline was given intravenously to these subjects.

Hypertensive patients on a low salt diet had depressed GFR, RBF and Tm_{PAH} values (Chasis *et al.*, 1950).

Sodium metabolism in pregnancy relative to kidney function has been inadequately investigated (Robinson, 1958; Lindheimer and Katz, 1970). These authors concluded that further research is needed in this area since now

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Animals:

High sodium chloride and low sodium chloride diets produce a number of changes in the kidneys.

A common finding associated with high dietary intakes of sodium chloride was renal hypertrophy (Krakower and Heino, 1947; Sapirstein *et al.*, 1950; Auerbach *et al.*, 1953; Fregly, 1960; Dahl and Schackow, 1964; Hall and Hall, 1966). A number of pathological changes were evident in kidneys from animals on a high sodium intake. Glomerular changes in the rat included hypertrophy, lipid deposition and degeneration (Meneely *et al.*, 1953b). Associated with these glomerular changes were edema and polyuria (Meneely *et al.*, 1953a). Krakower and Heino (1947) reported glomerular hypertrophy and glomerular and tubular degenerative changes in kidneys from chickens on a high sodium chloride intake. Tubular dilation and deaths from uremia occurred in rats on a chronic excess salt ingestion (Dahl and Schackow, 1964). Among the most extensive renal lesions observed with a high salt diet were reported by Auerbach *et al.* (1953) in rats fed a diet of 7.0 to 9.8% NaCl. Abnormalities included azotemia, enlarged glomeruli with swelling and

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vacuolation, and dilated tubules. These were present in 15% of the animals studied (Auerbach *et al.*, 1953).

Hypertension was a consistent finding in animals exposed to a high salt diet (Meneely *et al.*, 1953a, 1953b; Auerbach *et al.*, 1953; Dahl and Schackow, 1964). The relationship of the hypertension to the renal changes observed, especially the glomerular changes, has not been differentiated.

A number of pathological lesions were reported in rats fed simulated Japanese diets (Hilker *et al.*, 1965). Whether the sodium content or some other factor in the diet was responsible for the lesions was not determined (Hilker *et al.*, 1965).

Sodium deprivation also produced considerable renal damage (Cuttino *et al.*, 1948; Marx and Deane, 1963; Wardlaw and Pike, 1963; Ganguli *et al.*, 1969a, 1969b). Renal hemorrhage and dilated tubules (Cuttino *et al.*, 1948), decreased proximal tubular lumens (Marx and Deane, 1963), and juxtaglomerular alterations (Ganguli *et al.*, 1969a, 1969b; Marx and Deane, 1963; Wardlaw and Pike, 1963) occurred in rats on low sodium diets.

Magnesium

Humans:

Magnesium deficiency in man was recently reported (Flink, 1956; Vallee *et al.*, 1960); however, renal changes associated with this deficiency in man were not described.

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Magnesium deficiency in infants with kwashiorkor has also been reported (Montgomery, 1960), but no effect of a magnesium deficiency *per se* on kidney function was investigated.

Animals:

Rats fed a magnesium deficient diet developed calcification of soft tissues, particularly renal tissues (Tufts and Greenberg, 1936; Watchorn and McCance, 1937; Greenberg *et al.*, 1938; Sullivan and Evans, 1944; Hess *et al.*, 1959; McAleese and Forbes, 1961; Welt, 1964; Schneeberger and Morrison, 1965; Jacob and Forbes, 1969; Farnell and Whitehair, 1971). On the contrary, however, Schrader *et al.* (1937) did not observe calcification in rats fed a magnesium-deficient diet for 34 days.

KK mice (unlike ICR, C57BL and CFI mice) showed renal calcification when fed a low magnesium, high phosphorus diet (Hamuro *et al.*, 1970). Therefore, Hamuro *et al.* (1970) suggested that there was a strain difference in susceptibility to the renal calcification produced by the magnesium-deficient diet. Magnesium deficiency and calcification of renal tissue has also been produced in the dog (Featherston *et al.*, 1963), guinea pig (Pyke *et al.*, 1967) and cotton rat (Constant and Phillips, 1952).

A number of other degenerative changes affecting tubules and glomeruli were reported in the early studies of magnesium deficiency (Watchorn and McCance, 1937;

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Cramer, 1932; Sullivan and Evans, 1944). The diets used in some of these studies, however, were inadequate or imbalanced in respect to some nutrients other than magnesium. Also, the changes observed were described primarily as histological changes but no estimation of the functional capacity was made.

Other changes seen in magnesium deficient animals included nephrosis (Barron *et al.*, 1949); renal fibrosis (Barron *et al.*, 1949); increased proteinuria (Cramer, 1932; Greenberg *et al.*, 1938); glomerular degenerative changes (Cramer, 1932; Sullivan and Evans, 1944); and tubular degenerative changes other than calcification (Cramer, 1932; Schrader *et al.*, 1937; Greenberg *et al.*, 1938; Sullivan and Evans,1944; Hess *et al.*, 1959).

Whether a magnesium deficiency produces a polyuria (Cramer, 1932; Greenberg *et al.*, 1938; Smith *et al.*, 1962) or no change in urine volume (Manitius and Epstein, 1963; Schneeberger and Morrison, 1965) has not been established. Manitius and Epstein (1963) found that a potassium depletion can be induced in rats by a magnesium deficient diet with potassium levels that would normally be considered adequate. This result makes it more difficult to interpret much of the earlier work concerning the effect of a magnesium deficiency on renal function and histology.

Renal tubules in rats fed a magnesium-deficient diet with adequate potassium showed significant functional

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impairment which closely resembled that of potassium depletion (Smith *et al.*, 1962). Rats showed a 33% depression of concentrating capacity and a decreased response to an acid load when compared with rats on a control diet (Smith *et al.*, 1962).

The production of calcification of soft tissues, especially renal tissue by a magnesium deficient diet has provided a convenient tool for investigating factors that influence soft tissue calcification (Jacob and Forbes, 1969). L-thyroxine (Jacob and Forbes, 1969); Vitamin D depletion (Jacob and Forbes, 1970) and thyrocalcitonin (Farnell and Whitehair, 1971) prevented or lessened the severity of renal calcification normally seen in rats on a magnesium deficient diet.

Choline

Humans:

Choline may also play a role in maintenance of normal kidney structure in humans. Arends and Nieweg (1954) reported two cases of choline-like deficiency with renal and hepatic lesions closely resembling those produced experimentally in animals. Enlarged kidneys, tubular lesions, hemorrhage and casts were observed (Arends and Nieweg, 1954).

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Animals:

Acute choline deficiency in weanling rats is associated with hemorrhagic nephropathy accompanied by an increase in kidney weight, in part due to cellular proliferation (Griffth and Wade, 1939; Hartroft, 1948; Levenson *et al.*, 1968; Parks and Smith, 1968, 1969). Monserrat *et al.* (1968) suggested that, in weanling rats, lysosomal alterations are the outstanding pathological change in choline deficiency renal necrosis.

Renal hypertension developed in rats and dogs subjected to a choline deficiency (Hartroft and Best, 1949; Grollman and White, 1958). Nagler *et al.* (1968, 1969) reported that feeding diets low in choline to weanling rats produced an imbalance in vasoactive mediators due to a fall in tissue acetylcholine. They proposed that this led to vasospasm, ischemia, vascular rupture and the tubular necrosis seen in the nephropathy of acute choline deficiency (Nagler *et al.*, 1968, 1969).

Renal tubular degeneration and non-calcified, mucoid structures in the urinary bladder were seen in kidneys from rats subjected to diets marginal in choline and methionine (Newberne and Young, 1966). The "hemorrhagic kidney syndrome" of weanling rats was used as a bioassay to test the nutritional adequacy of various diets in supporting methylneogenesis for the synthesis of choline (Woodard, 1970).

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Vitamin B_{12} (Hawk and Elvehjem, 1953) and medium chain fatty acids in coconut oil (Zaki *et al.*, 1966) protected the young rat from hemorrhagic renal necrosis and fatty liver when fed a choline deficient diet. Short and long chain fatty acids aggravated the deficiency manifestations (Zaki *et al.*, 1966).

Protein

Humans:

Kidney function may be altered in kwashiorkor (Davies, 1956) and in other states of protein deficiency as previously discussed under the topic of malnutrition.

High protein levels had no adverse effect on renal function of humans according to Strouse and Kelman (1923). These workers fed 150 g of protein daily. Similarly, two Artic explorers who consumed an exclusive meat diet for 1 year had no albumin, casts or blood in urine specimens (Lieb, 1929). Furthermore, the urea clearance and phenol red excretion were normal. One subject followed by Newburgh *et al.* (1930) consumed 338 g protein daily. Albuminuria occurred after 6 weeks and hyaline and granular casts were evident after 7 weeks on this regimen. The urine was normal after the subject was fed a high carbohydrate diet for 10 days (Newburgh *et al.*, 1930).

The effect of the protein level on the urea clearance (C_{urea}) in man was extensively reviewed by Schmidt-Nielsen

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(1958). In general, the C_{urea} varies with the protein content of the diet. When the protein level is low, particularly over a prolonged period, the C_{urea} drops considerably (Cope, 1933; Goldring *et al.*, 1934; Nielsen and Bang, 1948, 1949; Pullman *et al.*, 1949). Increasing the protein level of the diet will increase the C_{urea} (Addis and Drury, 1923; Cope, 1933; Farr, 1936; Longley and Miller, 1942; Nielsen and Bang, 1948, 1949). Goldring *et al.* (1934) did not report an increased C_{urea} when the protein intake was increased. The control protein level, however, was 100 gm/day which would be considered by some researchers as a high level.

The maximum difference in C_{urea} between normal and low protein intakes was found at low urine flow rates (Schmidt-Nielsen, 1958). Whether the change in C_{urea} is due primarily to a change in glomerular or tubular function has not been established (Nielsen and Bang, 1948; Schmidt-Nielsen, 1958).

Bolourchi and co-workers (1968) presented data which suggest that the C_{urea} may be affected by the type, as well as by the dietary level, of protein.

The specific gravity of urine (Addis and Shevky, 1922) and the GFR (Nielsen and Bang, 1948; Pullman *et al.*, 1949) decreased when subjects were fed a low protein diet. The effective renal plasma flow (ERPF) was depressed 2% in females on a low protein diet (Nielsen and Bang, 1948).

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Conversely, the ERPF was increased 18% when the protein content of the diet was increased to 200 g/day (White and Rolf, 1948). An increase in ERPF was also reported by Pullman *et al.* (1949) when subjects were given 2.3 to 3.0 g protein per kg body weight per day. The GFR was also increased in persons on a high protein diet (White and Rolf, 1948; Pullman *et al.*, 1949).

Animals:

In animals, especially the dog, variations in protein intake have a greater effect on renal hemodynamics than in man (Schmidt-Nielsen, 1958).

Urea Clearance and Excretion: Factors affecting the urea excretion in dogs, seals, ruminants, rodents and other mammals was extensively reviewed by Schmidt-Nielsen (1958).

Urea clearance in the dog in the postabsorptive state (i.e., 18 hours postprandially) was increased on meat diets when compared with a low protein (cracker meal) or mixed diet (Jolliffe and Smith, 1931a, 1931b). Similar reports relating urea clearance to the protein level of the diet were published by numerous researchers (Rhoads *et al.*, 1934; VanSlyke *et al.*, 1934; Pitts, 1935).

Herrin *et al.* (1937) reported that the C_{urea} was increased when dogs were given casein, butter, glycine, alanine, glutamic acid, deaminated glycine, lactic acid, pyruvic acid, acetic acid and propionic acid. Gluconic acid did not elevate the C_{urea} in these dogs.

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Recently, workers demonstrated that the site of the accumulation of urea in the renal medulla was different in dogs on low and high protein diets (Truniger and Schmidt-Nielsen, 1964; Schmidt-Nielsen and Robinson, 1970). Furthermore, the excretion of urea is apparently dependent on the quality of protein as well as the amount of protein. When rats were fed casein or gluten so that nitrogen intakes and digestibility were equal, the urea excretion was increased in the gluten-fed group (Kiriyama and Ashida, 1964).

Glomerular Filtration Rate and Effective Renal Plasma Flow:

According to Smith (1951), the most complete study of the effects of protein on renal function in the dog was made by Moustgaard (1948) who showed that after a high protein meal the GFR and ERPF of dogs were increased. No alterations in the reabsorptive capacity for glucose in these animals was reported (Moustgaard, 1948). Smith (1951) reviewed in detail other studies completed by Moustgaard.

The GFR increased in dogs (Pitts, 1944; Ayer *et al.*, 1947) and in rats (Dicker, 1949) when the protein content of the diet was increased. Glycine when fed or infused produced increases in the GFR and ERPF in the dog (Friedman, 1946). Rats similarly treated showed no changes in the GFR or ERPF.

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No changes of an inflammatory or degenerative nature were observed microscopically in the kidneys of rats fed a 75% protein diet (Osborne *et al.*, 1923). Some minute tubular and glomerular changes occurred, but these were insignificant (Osborne *et al.*, 1927).

In contrast, rats fed less than a year a diet containing 40% or more liver had "granular" kidneys with degeneration of tubular epithelium and fibrosis (Newburgh and Curtis, 1928; Newburgh and Johnston, 1931). When rats were fed casein at a 75% level, however, only moderate tubular injury was evident at 16 months (Newburgh and Curtis, 1928). A partial explanation for the lesions produced by the liver may be its high content of nucleic acids (Newburgh and Johnston, 1931).

Medlar and Blatherwick (1937) reported that high protein diets produced chronic degenerative nephritis in rats. Although there were glomerular and tubular changes, the proximal convoluted tubules were normal in kidneys of these rats.

Polvogt *et al.* (1923) fed rats various proteins at a level of 31 to 41%. The kidneys of the original animals plus 5 of their subsequent generations (ages 5 months to 485 days)were examined histologically. Renal changes observed included hyalinization of glomeruli, congestion, degeneration of the tubular epithelium, glomerular adhesions, and hyaline casts. These researchers

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ascribed the renal damage to the excessive amounts of the end-products of protein metabolism (Polvogt *et al.*, 1923).

Osborne *et al.* (1927) observed glomerular and tubular changes in rats fed high protein diets. Renal tubules were dilated throughout the kidneys in rats fed diets containing 70% or more protein. Moise and Smith (1927) examined the remaining kidney of uninephrectomized rats fed an 18% or 85% protein ration. No anatomic evidence of significant renal damage in the 18% protein group was reported. The high protein group, on the other hand, showed significant glomerular and tubular changes. The lesions were conspicuous and relatively widespread, becoming progressively more marked with increased time on the diet. Glomerular changes included proliferation of the epithelium of Bowman's capsule with and without glomerular adhesions, fibrous thickening of Bowman's capsule, partial fibrosis of the glomerular tuft and some infiltration of round cells in fibrotic areas (Moise and Smith, 1927). Concomitantly, tubular changes reported were desquamation of the epithelial lining, some dilation and amorphous material within tubular lumina. Moise and Smith (1927) suggested that these changes were late manifestations of injury or irritation to which the kidney was subjected. In contrast, Addis *et al.* (1926) reported no difference in the kidneys of control and high-protein or high-cystine fed rats upon microscopical examination.

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Hogs fed a 42% crude protein diet had considerable renal damage when compared to controls fed 13.6% protein (Terrill*et al.*, 1952). Tubular changes in the high-protein fed group included dilation and amorphous deposits.

A deficiency of protein may produce alterations in kidney functions in animals. Dicker (1950) reported that the GFR and clearance of water in protein deficient rats was depressed when compared to controls. The concentration and total amount of sodium and chloride in the urine was decreased (Dicker, 1950). Dicker *et al.* (1946) reported that when rats were fed low protein diets (carrot or turnip diet), some histological changes in the tubules occurred. No glomerular abnormalities were observed. Tubular changes consisted of necrosis and calcification of the broad limbs of Henle. Protein-deficient rats responded less well to urinary concentration and dilution tests than the same animals on a standard diet. The total tubular excretory mass ($Tm_{Diodrast}$) of rats on the turnip diet was below control values. In contrast to normal rats, the GFR of the protein-deficient rats was significantly correlated with the rate of urine flow. Guggenheim (1956) reported that protein-deficient rats had normal GFR values, but abnormal responses to a water load.

Xylose Clearance: The glomerular clearance of xylose was increased in dogs fed meat or casein (Shannon *et al.*, 1932; Pitts, 1935).

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Hypertrophy: High protein levels produced renal hypertrophy in a number of species. These results are discussed in the section on factors affecting renal size.

In conclusion, the quantity and the quality of protein fed may affect renal function and structure.

Fat

When rats were fed diets virtually free of fat, kidneys were mottled with surface indentations (McAmis *et al.*, 1929). When Burr and Burr (1929) excluded fat from the diet of rats, kidneys were "mottled, spotted, abnormal." Furthermore, concretions in the bladder and hematuria were present. The kidneys from these rats were further examined by Borland and Jackson (1931). Grossly these were pale and larger than controls. No glomerular changes were observed. Lesions included tubular calcification, cortical tubular epithelium degeneration, increased intracellular fat in proximal and distal convoluted tubules, round cell infiltration and large quantities of "fatty or albuminous material" in the medullary area. Addition of lard at a dietary level of 2-20% prevented or cured these renal disorders. In the control animals, changes reported were round cell infiltration, some tubular degeneration and an increase in intracellular fat (Borland and Jackson, 1931). Later Rice and Jackson (1934a, 1934b) determined that the fat accumulation in the kidney was due to the high carbohydrate

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content of the diet and the general state of undernourishment and not to the "fatless" quality of Burr and Burr's diet (1929). With the advent of more chemically defined diets, the effects of fatty acid deficiency should be examined anew.

The choline requirement necessary to prevent renal necrosis was increased when rats were fed cocoa butter or corn oil at a 40% level (O'Neal *et al.*, 1961). Butter at this level, however, did not increase the lipotropic requirements.

Numerous calculi occurred in kidneys of rats fed methyl esters of fatty acids at a 10% level (Spining *et al.*, 1964).

Rats fed cyclized fish oil for 19 weeks had significantly lower urine specific gravities than animals fed a control diet. After 18 hours of water deprivation, the specific gravity of the urine of animals fed the fish oil was significantly less than that of the urine of control animals (Gottenbos and Thomasson, 1965). No histological changes in the kidney function were evident, however. Thomasson *et al.* (1966) found no disturbances of urinary concentrating capacity in rats fed hydrogenated fats (soya bean, linseed, olive or butterfat). Furthermore, the urine aspartate transaminase activity, which is increased in renal pathological conditions, was lower in the animals given the hardened oils than in controls fed unhardened soya-bean oil.

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Humans:

Herrin and Nicholes (1940) reported that the C_{urea} was increased in humans given vitamin A supplements for 2-3 weeks with a maximum response at 8 weeks. Furthermore, these researchers suggested that individuals with excessive subcutaneous fat are most likely to show an enhancement of the C_{urea} with vitamin A administration.

Similar results were reported by Taylor *et al.* (1943). Human patients given 100,000 to 400,000 I.U. of vitamin A for 5-90 days had increased values for GFR and renal plasma flow. These workers suggested that large doses of vitamin A could be used therapeutically in patients with degenerative renal diseases.

Animals:

Herrin and Nicholes (1939) observed that diets containing 150 g of butter or cod liver oil produced urea clearances in dogs that were 147 and 130% of control values. When vitamin A was given at a level of 50,000 I.U. daily the C_{urea} increased from 41 to 94% in a week. The maximum increase occurred after vitamin A supplementation for 96 days. In contrast to these findings, Bing (1943) found no change in the GFR, ERPF, Tm_{PAH}, or $Tm_{Diodrast}$ of dogs given daily 5,000 to 50,000 I.U. vitamin A orally. When 200,000 I.U. were added to the diet daily, a significant increase in Tm_D or Tm_{PAH} occurred. The GFR and ERPF were

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moderately increased with the filtration fraction remaining unchanged (Bing, 1943).

Rats when given high doses of vitamin A had increased values for GFR, RPF and $Tm_{Diodrast}$ (Dicker and Heller, 1946). Corcoran and Page (1947) reported that when crude extracts containing 2000 I.U. of vitamin A were injected to rats daily intramuscularly, the Tm_{PAH} was increased 100%. These investigators found no enhancement of renal function when crystalline vitamin A was given and concluded that the changes previously reported to be due to vitamin A were due to some other factor in the commercial concentrates. Corcoran and Page (1947) did not explain what factor in the butter and cod liver oil fed by Herrin and Nicholes (1939) was responsible for the increased C_{urea}. Herrin and Nicholes (1939) found that vitamin D was not responsible.

Vitamin C

Renal function was examined in 14 patients with florid scurvy by Eales (1956). The GFR was slightly reduced. A significant decrease in the ERPF was reported. The most striking change was a decrease in the effective renal blood flow (ERBF). The ERBF was related to a lower hematocrit primarily, but also to a diminution in the ERPF. The total excretory mass as measured by Tm_{PAH} was normal in 2 subjects and slightly decreased in a third (Eales, 1956).

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Vitamin D

An excess of vitamin D can produce hypercalcemia, hypercalciuria, and metastatic calcification in the kidney. This can lead to fatal or reversible renal failure. Dietary imbalances, such as a high-calcium, low-phosphorus diet, can also lead to renal disorders (Kark, 1968).

Vitamin D when administered over several weeks to dogs did not influence the C_{urea} (Herrin and Nicholes, 1939). When high levels of vitamin D were fed, the C_{urea} was depressed 42%.

Vitamin E

According to Herrin and Nicholes (1939) high levels of vitamin E were ineffective in altering the C_{urea} in dogs.

Kidneys from vitamin E deficient rats underwent rapid postmortem autolysis (Moore *et al.*, 1958; Emmel and LaCelle, 1961; György *et al.*, 1966). Vitamin E, choline, methionine and vitamin B_{12} were active in preventing this tendency to rapid autolysis (György *et al.*, 1966). Emmel and LaCelle (1961) found that the increased rate of post-mortem autolysis in the kidney was always preceded by a decrease in the renal tocopherol content.

The incidence and morphology of centrioles in the proximal convoluted tubules of rat kidney were altered by the dietary content of vitamin E (Hess and Menzel, 1968). Whether this would affect proximal tubular functional changes was not determined.

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B Vitamins, Calcium, Phosphorus and Others

Hamar (1940) reported that the tubular reabsorption of glucose was decreased in dogs fed a vitamin B_1 deficient diet.

Rats suffering from a lack of pantothenic acid, pyridoxine and riboflavin had depressed values for GFR and a water dilution test (Guggenheim, 1956). These returned to normal when the deficient animals were given ACTH or cortisone. Thiamine deficient rats also exhibited a delayed diuretic response to a combined inulin-water load, however, these were not restored when ACTH or cortisone was given (Guggenheim, 1956).

Pyridoxine deficiency in the rat was associated with a number of changes in the kidneys. These included hypertrophy (Agnew, 1951; Seronde, 1960), calcium or oxalate deposits (Agnew, 1951; Gershoff and Andrus, 1961) and casts (Agnew, 1951). Other workers, however, reported that when the pathologic manifestations due exclusively to pyridoxine deficiency were separated from the effects due solely to undernutrition in rats of similar sex, age, and weight, pyridoxine deficient animals had no renal hypertrophy or lesions (Wirtschafter and Walsh, 1964).

The effect of folic acid on producing renal hypertrophy is discussed in the section on factors affecting renal size.

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Calcium metabolism and its relationships to the kidneys of humans were reviewed by Kushner (1956). A number of diseases associated with hypercalcemia and metastic calcification produce renal damage. Calcium is a component of most primary urinary calculi.

Calcification of the kidneys was produced when rats were fed diets high in calcium, phosphorus and phosphoric acid for 3 weeks (Ham, 1940). High levels of phosphate also produced damage in kidneys of rats (MacKay and Oliver, 1935; McFarlane, 1941).

Addis and co-workers (1926) found a number of changes in rats fed acid (calcium chloride) or alkali (sodium bicarbonate) diets. The protein excretion of rats on the acid diets was one half that of controls. Significant hematuria and hydronephrosis occurred in rats on the alkali diet (Addis *et al.*, 1926).

Overweight and Obesity

Humans:

The incidence of cardiovascular-renal deaths in overweight persons is significantly higher than in those of normal weight (Mayer, 1968). The existence of renal and cardiovascular abnormalities among the obese appears to be an important factor responsible for the shortened life expectancy of this group according to Ross (1960).

The effects of overweight on kidney function have been investigated less than most other areas. As early

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as 1925, Dublin *et al.* reported that albuminuria was more frequent in overweight persons than those of normal weight. This was emphasized by Armstrong *et al.* (1951) who reported that statistical studies of periodic health examination data showed that the proportion of persons with albuminuria and glycosuria of significant degree was higher among overweight persons than among average and underweight persons. The occurrence of granular and hyaline casts was "noticeably greater" among overweights (Dublin *et al.*, 1925). Chronic and acute nephritis were also increased in the overweight (Dublin *et al.*, 1925: Britten, 1933).

The hazardous effects of perirenal fat deposition in the obese has been emphasized by Weil (1955). The proposed that in excessive overweight "large deposits of fat cover the viscera and fat infiltrates the parenchyma of vital organs." These morphological changes then could be reflected in a wide variety of physiological aberrations according to Weil (1955). On this basis, perirenal fat deposition in excessive amounts could lead to abnormal kidney function.

Table 1 is a summary of reports of renal function in obese patients. The purpose of most of the investigations cited was not related to determining kidney function; therefore, some of the data are incomplete. It is apparent from a number of studies that weight loss improves

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renal function in obese subjects (Weil, 1955; Lillington *et al.*, 1957a, 1957b).

The glomerular filtration rate (GFR), renal blood flow (RBF) and filtration fraction (FF) were significantly depressed in obese subjects (Bansi and Olsen, 1959; Olsen *et al.*, 1961). Olsen and his co-workers (1959; 1961), as well as others (Bittnerova *et al.*, 1968), have reported that obese persons have disturbances in water metabolism. A recent review (Gordon, 1970) points out that the obese are unable to excrete both salt and water normally. Bansi and Olsen (1959) suggested that in obese patients inadequate renal blood flow might be an essential factor in these disturbances. Triiodothyronine (T_3) restored the GFR, RBF, and FF to normal in obese patients (Bansi and Olsen, 1959; Olsen *et al.*, 1961). The mode of action of T_3 is not apparent since the extracellular fluid volume was normal in these patients both before and after T_3 treatment. Some evidence suggests that antidiuretic hormone (ADH) and aldosterone may be related to these disturbances. This suggestion is based on the increased blood levels of ADH (Bansi and Olsen, 1959; Olsen *et al.*, 1961) and increased aldosterone levels (Gordon, 1970) in obese subjects.

German workers recently reported somewhat different results in obese women. Bittnerova *et al.* (1968) found that the GFR was normal in their obese patients. The RFB and renal plasma flow (RPF), however, were depressed.

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Furthermore, upon water loading 53% of the obese subjects had a retarded excretion. The absolute values of the body water spaces were increased in the obese. These workers did not find differences in ADH plasma activity between obese and control subjects, however (Bittnerova *et al.*, 1968).

In conclusion, there is evidence to suggest that some renal functions are altered in obese humans. However, the degree of alteration and the mechanisms responsible for these alterations are not agreed upon by all investigators.

Animals:

The association of kidney damage with increased body weight in animals has been noted by several workers. Benedict *et al.* (1932) observed "congested and large" kidneys in an Osborne-Mendel rat that had reached a maximum weight of 822 g. Kidney lesions were also reported in the first hypothalamic obese rats (Brobeck *et al.*, 1943). The obese rats when killed 6 to 9 months after hypothalamic lesioning had advanced kidney disease and excessive proteinuria. These workers observed urinary casts, hematuria and histological glomerular and tubular changes. They called this condition "chronic glomerulonephritis." Kidney damage in hypothalamically obese rats has been confirmed by a number of workers (Brooks and Lambert, 1946; Stevenson, 1949; Kennedy, 1951, 1957; Long, 1957; Hausberger *et al.*, 1964).

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Kennedy (1951, 1957) concluded that the lesions observed in the hypothalamic obese rats were identical to those occurring spontaneously in old rats.

Considerable renal damage was observed in obese animals between 12-15 months of age, while no renal abnormalities were seen in control animals until 21 months. Later, Kennedy (1960) stated that if the protein in the diet had been increased from 15% casein to 20-25% casein, the lesions in the obese animals would have developed more rapidly. Goldblatt (1947) also observed that the changes in renal tissues were the same in obese younger animals as those in older normal weight animals.

The clearance of creatinine was depressed in hypothalamic obese rats (Stevenson, 1949). Other changes included a retardation in plasma flow and inability to respond to a water load (Stevenson, 1949). Similarly Long (1957) reported that albuminuria, hematuria and renal hypertrophy occurred in hypothalamic obese rats. He observed histological damage to the kidneys including hyalinization and fibrosis of the glomerulus, dilated tubules with thin epithelium and filled with amorphous material.

In rats made obese by purely dietary means, kidney abnormalities have been seen by several workers. Barboriak *et al.* (1958) observed tubular lesions in rats fed high fat diets. Preliminary studies at NIH (Yamamoto *et al.*, 1959) indicated an increase in

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proteinuria in obese rats, with males showing a greater increase than females. These workers attributed the increased proteinuria to an increase in the excretion of proteins with the same mobility as albumin and γ-globulin of serum. Craig (1952), using rats from the NIH colony, observed a statistically significant difference between the absolute kidney weights of obese and nonobese animals, but reported an absence of structural and functional alterations upon gross observation although large deposits of fat surrounded the kidneys. Naimi *et al.* (1965) found no abnormalities in kidney histology when rats became obese on a high-fat (butter) diet. These workers, however, looked at only 6 males at 6 months of age.

Rats with unrestricted feed intakes may also have an increased incidence of renal lesions. Berg and co-workers (Berg and Harmison, 1957; Berg, 1960; Berg and Simms, 1960) found chronic nephrosis practically nonexistent in old growth-retarded rats, but found a high incidence and increased severity of glomerular lesions in animals with unrestricted feed intakes. Bras and Ross (1964) found that in normal rats the shortest life expectancy was associated with *ad libitum* food intake and concomitantly the highest incidence of progressive glomerulonephrosis.

Genetically obese mice and rats also show considerable renal damage. The hyperlipemic "fatty" rat is frequently nephrotic (Zucker and Zucker, 1962; Zucker, 1965, 1967).

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The kidneys were hypertrophied and frequently contained kidney "sand" or stones (Zucker and Zucker, 1962). Microscopically, kidney lesions included thickened glomerular basement membranes, extensive tubular plugging and dilation and progressive replacement of nephrons by connective tissue (Zucker, 1965). Proteinuria and hypoalbuminemia frequently occurred especially in the males (Zucker, 1965, 1967). These renal lesions contributed to the shortened life span of the "fatty."

The *ob/ob* hereditary obese mice also exhibited renal lesions including lipohyalin deposits in the glomerulus (Hellman, 1965).

When obesity was produced in mice by injections of goldthioglucose (GTG) a number of changes in renal size and function were reported according to several workers (Waxler and Enger, 1954; Larsson, 1957). The kidneys were normal histologically in mice examined by Brecher and Waxler (1949) and Drachman and Tepperman (1954). The kidneys examined by Brecher and Waxler (1949) however, were from animals killed 14 weeks after GTG-injections. The long term effects of GTG-obesity on the kidney are not known.

In conclusion, obesity produced in animals by a number of different procedures is associated with abnormalities of renal structure and function. In fact, some workers have suggested that the renal damage contributes in large part to the premature death of obese animals.

Table 1. Summary of reports of renal function in obese subjects.[a]

Subject and Weight, etc.	Measurements or Comments Re: Renal Size, Function and Histology	Reference

Table 1. Summary of reports of renal function in obese subjects.[a]

Subject and Weight, etc.	Measurements or Comments Re: Renal Size, Function and Histology	Reference
29 boys and 21 girls with "endocrine" obesity	Normal BUN and creatinine Salt and H_2O retention High blood Cl^- and uric acid	Gordon, 1937
200 pregnant subjects with body weights >200 lbs.	Casts Albuminuria in 1/3 during early pregnancy	Matthews and DerBrucke, 1938
1 male - 380 lbs. → 290 lbs.	380 lbs→290 lbs 24hr urine protein 9.72 gm 0.13 gm PSP excretion at 30 min. 26% 41% Maximum urine concentration 1.019 1.027 BUN 20.5mg% 10 mg%	Weil, 1955
1 male - 364 lbs.	"Golden granules on the cut surface of the kidney" Fatty infiltration of tubules	Counihan, 1956

Table 1. (cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
[illegible]	Albuminuria-trace.	Carroll, 1956

Table 1. (Cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
1 male - 371 lbs.	Albuminuria-trace. PSP-62% excreted in 2 hours Patient reduced to 213, 2^+ albuminuria and PSP-55% At death, kidneys were grossly normal. Small black friable stones were in calyces of both. (B.W. 246 lbs)	Carroll, 1956
1 male - 370 lbs.	Albuminuria which disappeared when wt. decreased to 300 lbs.	Lillington *et al.*, 1957a
1 female - 427 lbs.	Albuminuria Increased BUN level Returned to normal at body weight of 251 lbs.	Lillington *et al.*, 1957a
1 male - 430 lbs.	Albuminuria-3^+ Microscopic hematuria BUN-normal Cylindruria	Lillington *et al.*, 1957b
1 male - 335 lbs.	Albuminuria-2^+ BUN-72 mg%	Lillington *et al.*, 1957b

Table 1. (Cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
	Urinalysis-normal	Lillington *et al.*, 1957b

Table 1. (Cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
1 male - 240 lbs.	Urinalysis-normal BUN-72 mg%	Lillington *et al.*, 1957b
1 male - 300 lbs.	Albuminuria-2^+ BUN-normal	Lillington *et al.*, 1957b
1 male - 370 lbs.	BUN-21 mg%	Lillington *et al.*, 1957b
1 male - 273 lbs.	Albuminuria-3^+ BUN-normal	Lillington *et al.*, 1957b
↓ 225 lbs.	Albuminuria-2^+	
↓ 242 lbs.	Albuminuria-2^+	
↓ 190 lbs.	No albuminuria BUN-26 mg%	
1 female - 5'2" 427 lbs.	Albuminuria-4^+ BUN-64 mg%	Lillington *et al.*, 1957b
↓ 251 lbs.	No albuminuria BUN-normal	
1 female - 262 lbs.	Normal urine function	Gϕtzsche and Peterson, 1958
1 male - 325 lbs.	Normal urine analysis	Fulmer, 1958

Table 1. (cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
	Kidney function-"somewhat	Berlyne, 1958

Table 1. (Cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
1 male - 364 lbs.	Kidney function-"somewhat impaired" BUN=60 mg%	Berlyne, 1958
1 patient - 404 lbs. 5'9" hgt.	"Normal urinalysis"	Smith, 1959
27 obese patients - 15-56 years (sex & weight not given)	Renal plasma flow-decreased significantly Glomerular filtration rate decreased significantly Filtration fraction decreased significantly	Bansi and Olsen, 1959
15 males, 35 females 14-70 years, mean excess weight of 223 lbs.	16 PSP clearances performed-all normal All BUN's and urinalyses within normal limits No history of urinary symptomatology	Alexander *et al.*, 1962
20 obese patients 16 females, 4 males 15-54 years of age	No changes in BUN, serum creatinine, uric acid, or urinary 17-ketosteroids or 17-hydroxycorticosteroids with weight loss	Politzer and Bersohn, 1963

Table 1. (cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
	Larger and heavier kidneys	Alexander, 1963

Table 1. (Cont'd)

Subject and Weight, etc.	Measurements or Comments	Reference
Subjects 110-250 lbs. overweight	Larger and heavier kidneys than normal No increase in renal blood flow	Alexander, 1963
13 obese females	Clearance of urea and endogenous creatinine decreased during fasting Increased urine pH with fasting	Rapoport *et al.*, 1965
2 obese subjects 1. 132kg-158 cm 1. 112kg-160 cm	With fasting, decreased GFR and increased plasma creatinine	Smith *et al.*, 1969
48 pregnant subjects-body weight>250 lbs.	BUN=10.1 mg% 5 times expected rate of pyelonephritis	Tracy and Miller, 1969

[a]The purpose of most of the investigations cited was not related to determining renal function. Some of the observations reported were made in a general clinical examination.

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PART II. Histopathologic description of renal lesions in rats fed a high fat diet through 45 weeks of age.

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INTRODUCTION

A number of manipulations, including dietary ones, produce changes in kidney structure, size and function in the rat. In addition, the histological appearance of the kidney is affected by age. Lesions have been described in kidneys from rats made obese by hypothalamic lesions, genetic selection, and dietary procedures. The histological changes occurring in the kidneys of rats made obese by feeding a high fat diet have not been described. The objectives of this investigation were to describe the histological changes in kidneys of male rats as they became obese and to compare these changes with those normally occurring in rats with aging.

LITERATURE REVIEW

According to Snell (1967), literature dealing with the influence of diet on renal lesions is "somewhat contradictory and difficult to assess." These contradictory results could reflect differences in the inherited characteristics of the strain of rats, differences in basal dietary components and differences in criteria for evaluation of the sections.

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The rat is remarkably resistant to the development of spontaneous renal tumors. These rarely occur in rats fed control rations (Snell, 1965, 1967). Curtis *et al.* (1931) reported that of 24512 rats of 7 strains investigated, only eleven females and 1 male had renal tumors. In another study, the incidence of spontaneous renal tumors was only 5 in 468 animals (Ratcliffe, 1940). Kidney neoplasms occurred in 4 of 1342 rats; however, the ages of the 3 males and 1 female with these tumors exceeded 2 years (Gilbert and Gillman, 1958). When rats were irradiated at 230 or 320 rads, renal tumors developed in 41% and 43% of the animals respectively, while none occurred in the control animals (Rosen *et al.*, 1962). A single pleomorphic renal tumor (Babcock and Southam, 1961) and a renal adenosarcoma (Lillie and Engle, 1935) also have been observed in rats.

Changes in Renal Function and Histology with Age

Renal function and histology are related to the age of an animal or human.

Humans:

The newborn infant has impaired renal function (Edelmann and Spitzer, 1969). At the other end of the life span, that of old age, the kidney is also less capable of functioning maximally. In subjects from 20 to 90 years of age, the glomerular filtration rate (GFR), the effective renal plasma flow (ERPF) and the excretory capacity

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or tubular maximum for Diodrast ($Tm_{Diodrast}$) decreased 46, 53 and 44%, respectively (Davies and Shock, 1950; Shock, 1952, 1956). There was a gradual diminution of the urea clearance and a progressively increased BUN with advancing age (Shock, 1952). In addition, the excretory capacity of the renal tubules for *p*-aminohippurate (PAH) (Tm_{PAH}) and for glucose ($Tm_{Glucose}$) decreased as age increased (Shock, 1952). The decreased ERPF cannot be explained fully on a basis of structural changes in renal blood vessels as renal arterioles in the aged kidney were capable of dilating when subjected to a standardized pyrogen test (McDonald *et al.*, 1951). The ability of the distal tubule of the older individual to perform osmotic work when provided with a standardized amount of antidiuretic hormone (ADH) was also impaired (Miller and Shock, 1953). On the basis of the observed changes in a number of kidney functions and the constant ratio between GFR and Tm over 7 decades, Shock (1952) suggested that the nephron loses its function as a unit.

In contrast to the other functions described above, normal acid-base balance was maintained in spite of depressed kidney function (Shock and Yiengst, 1950). Acid-base balance, however, is not regulated totally by the kidney.

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The kidneys from newborns of a number of species are less capable of transporting organic acids and bases than those from mature animals (Williamson and Hiatt, 1947; New *et al.*, 1959; Rennick *et al.*, 1961; Hirsch, 1970). A number of other renal functions are immature in the newborn (McCance & Widdowson, 1954, 1955, 1957). The kidney of the newborn rat contains a nephrogenic zone with malpighian corpuscles at various stages of formation and incompletely formed proximal tubules (Bogomolova, 1966). Baxter and Yoffey (1948) suggested that only a proportion of renal tubules are fully functional in the rat at birth. Other workers (Enesco and LeBlond, 1962) found that the number and size of renal cells increased in the rat kidney from 7 to 95 days of age. Kunkel (1930) reported that the number, size and capillary surface of glomeruli were more closely related to body surface than any other measurement. There is a progressive decrease in the number of nephrons in the rat kidney after middle age (Arataki, 1926).

Kidney cortical slices from old rats were less able to transport PAH and α-aminoisobutyric acid (AIB) than younger adults (Adams and Barrows, 1963; Beauchene *et al.*, 1965). Old rats were less able to concentrate their urine than young adults (Dicker and Nunn, 1958). An intracarotid injection of hypertonic NaCl produced a smaller antidiuretic effect in old rats. Dicker and Nunn (1958)

suggested that this defect was of renal rather than of neurohypophyseal origin in contrast to earlier reports by Friedman and Friedman (1957).

Histological changes in kidneys from old rats included disintegration of mitochondria in the epithelium of degenerating nephrons, hyaline droplets within tubular cells and hyaline casts within tubular lumens (Bogomolova, 1966). Kennedy (1957) has suggested that although in old age the kidneys differ histologically from the normal kidney of youth, it is not always a simple task to distinguish the end results of senile atrophy from those of pathological injury. A review of the literature on the effects of various diets on renal histology substantiates Kennedy's suggestion.

Saxton and Kimball (1941) reported renal lesions in male Osborne-Mendel rats from 259-620 days old fed regimens including high protein and low protein levels. Forty-four percent of the rats dying natural deaths showed lesions indicative of "chronic nephrosis." The histological observations included hyaline casts in tubules,dilation of glomerular capsules, and hyaline thickening of the basement membranes. Not all tubules and glomeruli, however, were affected even in the most severe cases. Saxton and Kimball (1941) suggested that these lesions constituted a spontaneous disease of rats and were not specifically produced by diet. The lesions, however, were modified by diet as they were more severe in

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animals fed high protein diets than in those fed low protein diets, in rats fed casein rather than liver, and in rats allowed food on an unrestricted basis when compared to those on a restricted regimen (Saxton and Kimball, 1941).

Similar lesions in kidneys of rats were described by numerous workers (Moise and Smith, 1927; Blatherwick and Medlar, 1937; Wilens and Sproul, 1938; Simms and Berg, 1957; Andrew and Pruett, 1957; Kennedy, 1957; Gray, 1963; Kennedy and Parker, 1963b; Foley *et al.*, 1964; Bras and Ross, 1964; Gray and Purmalis, 1965; Bras, 1969), and classified under a variety of names including nephrosis, nephritis, pyelonephritis and glomerulosclerosis. Table 2 is a compilation of selected reports of spontaneous changes in renal histology observed in rats. It is apparent from this table that a variety of terms have been used to classify similar changes.

Kidneys from rats made obese by a number of procedures show alterations in structure microscopically. Kennedy (1951) described the changes in kidneys from hypothalamic obese rats as follows:

> "The damage to the kidney appears to fall primarily on the tubules. The epithelium becomes atrophic and the lumen is full of hyaline debris. There is relatively little glomerular or vascular damage until a late stage, and although the animals are sometimes hypertensive, this is not constant. Histologically it has the appearance of pyelonephritis, which Goldblatt has shown to be common

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> in some strains of rats. But none of our controls have shown kidney lesions, and we have found no sign of infection at any stage in the fat animals. Although it might be tempting to regard it as due to a relative deficiency of lipotrophic substances, the slight degree of liver involvement is against this."

He suggested that the obesity could be a non-specific stress and that the kidney damage was secondary to over-activity of the adrenals (Kennedy, 1951). The adrenals in these animals were hypertrophied. Brobeck, Tepperman and Long (1943) observed these lesions in hypothalamically obese rats and called the condition "chronic glomerulo-nephritis." Kennedy, after confirming these lesions (1951, 1957, 1960), suggested that they were identical with those that occur spontaneously in senility.

Bras and Ross (1964) reported that restriction of intake, whether of protein alone, of carbohydrate alone or of both had a beneficial effect upon the incidence of spontaneous renal disease. The incidence was greatest in rats fed a commercial diet *ad libitum*. Obesity was common in these animals.

The type of fat used to produce dietary obesity could also influence the occurrence of renal lesions. György *et al.* (1966) reported that the renal changes in rats fed lard and cod liver oil were more severe than in those fed Crisco. Kaunitz *et al.* (1970) suggested that the minor *dif* ferences in various fats including their methods of processing could influence the incidence of renal lesions.

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The histological lesions observed by Kaunitz and co-workers (1970) included renal tubular calcification, atrophy or dilation of tubules by hyaline casts. Glomerular changes were mild and included thickening and calcification of Bowman's membrane. All of these lesions are the same as seen in secondary hyperparathyroidism (Kaunitz *et al.*, 1970).

Renal lesions can result from a variety of other dietary manipulations including a deficiency of choline (Hartroft, 1948), potassium (Schrader *et al.*, 1937; Follis *et al.*, 1942; Follis, 1943; Newberne, 1964), protein (Dicker *et al.*, 1946) and magnesium (Watchorn and McCance, 1937; Cramer, 1932; Sullivan and Evans, 1944).

Table 2. Selected reports of spontaneous changes in renal histology in rats.

Table 2. Selected reports of spontaneous changes in renal histology in rats.

Animals	Description	Reference
Control rats >21 months old	1) Increase in the number of epithelial cells of occasional tubules 2) Hyperphasia of parietal epithelium of Bowman's capsule 3) Wide-spread tubular dilation and atrophy 4) Glomeruli-fibrosed, capillary tuft bloodless and adhered to Bowman's capsule, thickened glomerular basement membrane 5) Some round cell infiltration 6) Few, if any, vascular changes 7) "Chronic pyelonephritis"	Kennedy, 1957; Kennedy and Parker, 1963
50 Wistar rats (Senile)	1) Colloid-like material precipitated in the tubules 2) Clear areas, probably caused by deposits of fatty material, in the media of arteries 3) Little fibrosis of glomeruli 4) Malpighian corpuscles have pronounced basement membrane and a greater dilation of glomerular capillaries 5) Glomeruli of senile rats were larger than those of young (75-100 day) and middle-age (300 day) rats	Andrew & Pruett, 1957; Andrew, 1971
Male rats (Senile)	1) "Chronic nephrosis and glomerulonephritis" 2) Dilation of tubules with flattened epithelium	Simms and Berg, 1957

Table 2. (Cont'd)

Table 2. (Cont'd)

Animals	Description	Reference
	3) Glomeruli-atrophied, hyalinized 4) Increase in connective tissue	
BHE male rats	1) "Nephrosis" or spontaneous kidney disease 2) Hyaline casts in tubules 3) Glomerular crescent formation 4) Tubular hyperplasia	Durand *et al.*, 1964
Aging female breeders (12-15 months old)	1) "Nephrosclerosis" 2) Small areas of focal lymphocytic infiltrates 3) Hyaline eosinophilic casts, especially in collecting tubules 4) Glomerular changes including thickening of basement membrane, edema of capillary loops, shrinkage and partial adhesion of glomerular loops to Bowman's capsule	Wilgram and Ingle, 1959

Table 2. (Cont'd)

Table 2. (Cont'd)

Animals	Description	Reference
Charles River SD male rats 21-1600 days old	1) "Progressive glomerulonephrosis" (PGN) 2) Glomeruli-increased density in glomerular tufts, basement membranes thickened some "crescent" formation 3) Tubules - casts, distended tubules, flattened epithelial cells 4) Interstitial tissues-condensation, some foci of lymphocytic cells 5) Blood vessels - few changes	Bras and Ross, 1964 Bras, 1969
Sprague-Dawley (Upjohn) rat	1) "chronic progressive degeneration" of the kidneys 2) Cast formation in the distal and later in the proximal portions of the tubules 3) Mild focal hyperplasia of the epithelium of the proximal convolutions 4) Thickening of basement membranes of the glomerular tufts, of Bowman's capsule and of the proximal convolutions 5) Late associated influx of lymphocytes among interstices of affected tubules.	Gray, 1963; Gray and Purmalis, 1965

Kidney Size and Factors Influencing It

A number of factors may influence the size of the kidneys. Increased kidney weight may result from an increase in cell number (hyperplasia), cell size (hypertrophy), intracellular material or a combination of these.

Renal growth may result from subjecting an animal to: partial nephrectomy, nephrectomy serum, ureteral ligation, intraperitoneal injection of a protein, a high sodium diet, a low potassium diet, NH_4Cl, folic acid, thyroxine, testosterone, cold, growth hormone and mineralocorticoids (Goss and Dittmer, 1969). A number of these will be discussed in detail.

Dietary Nitrogen

The amount and nature of the nitrogen in the diet may affect kidney weight. A diet with 75% protein of vegetable or animal origin produced kidneys which were 2 times the average weight of those from control rats (Osborne *et al.*, 1923, 1927). No microscopic or degenerative changes were observed in kidneys from rats fed these diets although the kidney weight/body weight (KW/BW) ratios were twice those of control rats (Osborne *et al.*, 1923, 1927). Other workers have reported increased kidney weights in hogs (Terrill *et al.*, 1952), piglets (Filer *et al.*, 1960), and dogs (Allen and Cope, 1942) with an increased dietary protein intake. Filer *et al.* (1960) reported increased kidney weights with increased protein

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content in the diet whether it was a high fat or low fat diet. A number of other workers have reported increased kidney weights with a high protein diet (MacKay *et al.*, 1926b, 1926c; MacKay *et al.*, 1928a, 1928b; MacKay and MacKay, 1931a, 1931b; Medlar and Blatherwick, 1937; Chanutin and Ludewig, 1939a, 1939b; Walter and Addis, 1939; McCay *et al.*, 1941).

Reid (1963) found that differences in casein levels from 20-70% produced no significant changes in kidney weights of guinea pigs. The kidneys of guinea pigs fed soy protein at a level of 30 to 70% protein were significantly heavier than those of guinea pigs fed soy protein at a level of 20 or 25% protein (Reid, 1963). Reid (1963), however, emphasized that comparisons between the rat and guinea pig as to effect of protein level of kidney size were not justified. She pointed out that the guinea pig studies dealt only with the early stages of growth (6 weeks) whereas most of the work with rats was with older animals (Reid, 1963).

The type of protein may affect the degree of hypertrophy. When rats were fed gelatin their kidney weights were significantly heavier than those of rats fed caseinogen or liver (Wilson, 1933). The mechanism for the gelatin effect on kidney weight has not been determined (Halliburton, 1969). The degree of compensatory renal hypertrophy after nephrectomy was directly proportional to the dietary protein content (Smith and Moise, 1927).

MacKay *et al.* (1926a, 1931) reported increased kidney weights in rats fed urea or urea plus protein. The hypertrophy was less than that produced by the same nitrogen consumption obtained by protein alone. Thus, the absolute nitrogen consumption did not explain the hypertrophy observed with an increased dietary protein intake. In contrast, glycine, glutamic acid and gluten produced increased kidney weights in proportion to the nitrogen consumed (Wilson, 1933). The type of protein or nitrogen source differed in these studies and may partially explain the apparent discrepancy in these results when compared to those of MacKay's group.

The hypertrophy observed in animals fed a high protein diet was apparently not associated with an increased urine volume. The increased urine volume is a concomitant of the high protein intake (Stier and Hayman, 1938).

The blood urea nitrogen (BUN) of rats increased with an increased protein intake. When the (BUN) 2/3 was plotted against the renal weight per 100 cm^2 of body surface the relationship was linear (MacKay *et al.*, 1928a).

A protein deficiency in the dam may affect the weights of the kidneys of rats *in utero*. Zeman (1967) reported that kidneys in newborn rats from dams fed an 18% casein diet during pregnancy were 1.014% of the body weight. When dams were fed a 6% casein diet during pregnancy, their offspring at birth had kidneys which were only 0.788% of the body weight (Zeman, 1967). Other workers have

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reported that kidney weights of older rats on diets devoid of protein are almost directly proportional to their endogenous protein metabolism (MacKay and Cockrill, 1931b).

Other Dietary Factors

Kidney weights were increased in potassium-deficient rats although body weights were less than those of controls (Follis *et al.*, 1942; Fuhrman and Brokaw, 1951; Muntwyler and Griffin, 1953; Brokaw, 1953). Deficiences of Vitamin B_6 (Seronde, 1960) and choline (Griffth and Wade, 1939; Parks and Smith, 1969) also produced kidney hypertrophy. Animals on a high sodium chloride intake of dietary origin (food or water) had significantly larger kidneys than control animals (Krakower and Heino, 1947; Sapirstein *et al.*, 1950; Auerbach *et al.*, 1953; Fregly, 1960; Dahl and Schackow, 1964; Hall and Hall, 1966). Whether the increased kidney weight in animals on a high sodium chloride intake is related to the concomitant hypertension in these animals has not been determined. A single injection of folic acid also produces renal hypertrophy (Threlfall, 1969; Hirsch and Hook, 1969).

When young rats were fed rations with increased levels of phosphate, kidney weights of these animals were increased (MacKay *et al.*, 1926a). Rats on a fat-free diet also had hypertrophied kidneys at ages 2.5 to 19 months (Borland and Jackson, 1931). There were no

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statistical differences in the weights of kidneys when adult female rats were fed a 68% sucrose, 18% casein, 10% vegetable oil diet or a 48% cornstarch, 30% egg white, 14% cottonseed oil diet (Peters and Krijnen, 1966). Wierda (1950) reported changes in kidney weights when animals were fed diets that were unbalanced in various nutrients. He reported kidney weights of animals fed a 25% casein, 69% lard diet were greater than those from animals fed a 14% casein, 83% cornstarch ratio.

Underfeeding or short periods of fasting also affected the kidney weights (Stewart, 1919; McCay *et al.*, 1939a, 1939b; Widdowson and McCance, 1956; Peters and Boyd, 1965; Brown and Guthrie, 1968). Stewart (1919) reported that underfeeding rats until age 11-22 days by repeated periods of fasting from the day of birth produced a 90% increase in the absolute kidney weight in males and females. The kidney weights were decreased in rats retarded by limiting caloric intake (McCay *et al.*, 1939a). Other workers reported that after two weeks of reduced food intake the changes in kidney weights decreased approximately in proportion to changes in body weight (Peters and Boyd, 1965, 1966).

Body Weight

Absolute kidney weights increased linearly as body weight increased in rats after a body weight of 50 gms (Hatai, 1913). Similar results were reported in rats by

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Webster *et al.* (1947) and Widdowson and McCance (1960). In contrast, Forster (1947) found no close relationship of kidney weights and body weights in rabbits.

The relative kidney weight or kidney weight/body weight ratio (KW/BW) ranged from 1.16 to 0.74 and decreased with increasing weight from 50 to 200 g (Webster *et al.*, 1947). Similarly, Widdowson and McCance (1960) reported that the kidneys of rats grew faster than the body as a whole initially and the KW/BW ratio exceeded 1. With increasing age the KW/BW ratio decreased.

An increase in the absolute renal weights occurred in obese mice whether the obesity was genetically transmitted (*ob/ob*), GTG-induced or produced by hypothalamic lesions (Marshall *et al.*, 1957). The KW/BW ratio decreased in these obese animals. Marshall *et al.* (1957) suggested that the renal enlargement was due to a nonspecific effect of the hyperphagia and obesity.

The kidney weight on a dry or defatted basis was not increased in proportion to increases in body weight as mice injected with GTG became obese (Waxler and Enger, 1954). When these GTG-obese mice were reduced to body weights equal to that of controls, the absolute weights of the kidneys were less than those of control animals (Waxler and Enger, 1954).

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Age

MacKay and MacKay (1927) concluded that in the albino rat the kidney weight had practically the same relation to body surface at all ages. Later these workers reported that the kidney weight to body surface ratio fell slightly with increasing age (MacKay and MacKay, 1934). They suggested that this was due to a decreased protein intake in relation to body surface in the older animals. Other workers reported that kidney weights were related more to body weight than to age (Widdowson and McCance, 1960).

In humans, kidneys at birth represented a higher proportion of the body weight than they did at any other age (Widdowson and Dickerson, 1960).

The effect of dietary protein on renal growth may be modified by age.

MacKay *et al.* (1928b) and MacKay and MacKay (1931a) reported that age up to 346 days had no influence on the linear relationship between the renal weight and protein intake. The absolute and relative increase in renal weight which followed a given percentage increase in protein intake, however, decreased as age increased (MacKay *et al.*, 1928b). From 346-400 days of age increased protein in the diet had little effect on kidney size in males (MacKay *et al.*, 1926b). In the female of all ages there was a linear relationship between the renal weight and protein intake (MacKay and MacKay, 1931b).

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Miscellaneous

Kidney weights of rats were influenced by strain and sex (Freudenberger, 1932). Eaton (1938) reported that sex and genetics influenced kidney weights of guinea pigs. Exposure to cold caused hypertrophy of kidneys in rats of both sexes (Emery *et al.*, 1940). Exercise affected kidney size in rats according to Bloor *et al.* (1968). When animals were given thyroxin (Walter and Addis, 1939) or thyroid (MacKay and MacKay, 1931c) renal weight was increased above that of control animals.

Chronic acidosis in rats also resulted in renal enlargement (Janicki, 1970). Constantinides (1951) reported that stresses (formalin injections, cold, forced exercise) for 20-48 hours duration caused an immediate enlargement of the kidneys. Dinitrophenol produced hypertrophy of the kidney in albino rats (Murphy, 1938).

Kidney weight may be influenced by a number of dietary and nondietary factors (Goss and Dittman, 1969; Halliburton, 1969). The mechanisms responsible for the renal hypertrophy produced by these has not been elucidated.

The objectives of this experiment were (1) to describe the lesions occurring in kidneys from animals made obese by dietary means, (2) to compare these lesions with those occurring spontaneously in rats and (3) to determine the influence of dietary obesity on renal weights.

MATERIALS AND METHODS

Animals

Osborne-Mendel rats bred in the Department of Foods and Nutrition were chosen for this study for two reasons: (1) They are relatively resistant to upper respiratory infection and are thus suitable for long term studies (Mickelsen *et al.*, 1955); (2) They attain a large body size and become obese, as measured by total ether-extractable body fat, when fed a 60% hydrogenated fat semi-synthetic diet (Mickelsen *et al.*, 1955; Schemmel *et al.*, 1969).

Within 48 hours after birth, litters were cut to eight pups by keeping all of the males and making the litter to eight with females. If less than eight animals were born, all were saved. Each litter was weaned at an average age of twenty-three days. Grain ration and water were available in the breeding cage at all times.

After weaning, males were paired but housed in separate wire-bottom cages in a temperature and light controlled room. One male of each pair was fed a grain ration (GR) and the other a semi-synthetic high fat ration (HF). Females were not used in this experiment.

Rations

The two rations fed in this study were developed by Smith (1969). They are modifications of a grain ration (Campbell *et al.*, 1966) and a semi-synthetic ration containing 60% fat (Mickelsen *et al.*, 1955). Composition of the modified rations are shown in Appendices C and D. The diets were altered by Smith (1969) so that animals on the grain ration or high fat ration would consume the same quantities of calcium, magnesium, and phosphorus per week. The calcium to phosphorus ration in these diets is similar to that recommended for the growing rat by the National Research Council (Committee on Animal Nutrition, 1962). The two rations and distilled water were available *ad libitum*.

Tissue Preparation

A total of 73 animals were used in this experiment. At predetermined ages (weaning, nine, fifteen, twenty-five, thirty-five and forty-five weeks), the animals, after being weighed, were killed instantly by using an overdose of ether in a closed chamber. Kidneys were removed from capsules, blotted, and weighed separately. Kidneys were cut transversely in 1.0 mm sections. Separate pieces were fixed in 4% acetate-buffered formaldehyde, modified Zenker's fixative, or Carnoy's fixative (Lillie, 1965; Armed Forces Institute of Pathology, 1968). After 12-24 hours, Zenker-fixed tissues were washed in running

tap water 8 hours, and then stored in 80% ethanol. The sections in Carnoy's solution were fixed for 6-12 hours and then placed in absolute alcohol. All samples were stored at room temperature. The entire cross sectional kidney slices were dehydrated, embedded in paraffin and cut at 5 microns. Hematoxylin-eosin stain was used routinely (Lillie, 1965). Special stains were applied where needed. These included Oil Red O, PAS, Alcian Blue, and Congo Red.

Parameters evaluated in histological sections of kidneys are shown in Appendix E.

Statistical Analyses

The paired Students "t" test was used to assess the significance of differences between group means. This test was also used to assess the differences in the weights of the right and left kidneys within the same age and dietary group.

An analysis of variance (Steel and Torrie, 1960) was used to delineate factors affecting kidney weight, body weight and the kidney weight/body weight ratio.

RESULTS

Body Weights:

Animals fed either ration increased in weight as they grew older, but animals fed the HF ration increased their weights at a faster rate than those fed the control diet (Table 3). By ages 25, 35, and 45 weeks, animals fed the HF diet weighed significantly more than animals fed the GR diet (25 and 35 weeks-$P<0.001$; 45 weeks-$P<0.05$). From gross observation, the HF animals contained considerably more carcass fat than the GR animals, particularly perirenal fat. The effect of age and diet upon body weight were each statistically significant ($P<0.01$) for all rats (Table 5). Furthermore, the interaction of diet and age was statistically significant ($P<0.01$).

Kidney Weights and Kidney Weight/Body Weight Ratios

In the HF fed group, the right kidney was heavier (15, 35 and 45 weeks-$P<0.02$; 25 weeks-$P<0.01$) than the left kidney at all ages (Table 3). There was no difference ($P>0.05$) in the weights of the two kidneys in the GR groups at any age (Table 3).

The total kidney weight was significantly greater in HF animals at 25 ($P<0.02$), 35 ($P<0.01$) and 45 ($P<0.01$) weeks than in GR animals (Table 3). The effect of diet was statistically significant ($P<0.01$) for all rats

(Table 5). Furthermore, the interaction of diet and age was statistically significant ($P<0.05$).

The kidney weight/body weight ratio (KW/BW) (Table 3) was significantly less in HF animals at all ages (15 weeks-$P<0.05$; 25 and 45 weeks-$P<0.001$; 35 weeks-$P<0.01$). Diet and age had a statistically significant effect on the KW/BW ratio at the 1% and 5% level of significance, respectively (Table 6). The interaction of diet and age was statistically significant ($P<0.05$).

Histopathological Examination

The incidence of lesions observed histologically in kidneys from GR and HF animals is shown in Table 7.

Kidneys of weanling animals (Figure 1) had small densely cellular glomeruli. The outer cortex had a nephrogenic zone with undifferentiated glomeruli and tubular cells. No inflammation or degenerative changes were observed in these kidneys.

At 15 weeks changes were observed in kidneys from GR and HF animals (Table 7). Kidneys from HF animals (Figure 2) had considerable glomerular damage. Tubules contained some cellular debris. Although there were degenerative glomerular changes in this kidney, there were areas with normal appearing glomeruli.

The incidence of different types of lesions was greater in the 15 week GR group than the HF group (Table 7). The lesions observed in the HF group were more widespread within the kidney.

Kidneys from GR animals at 25 weeks (Figure 3) had large, well-defined glomeruli. Tubules were relatively free of debris or secretion. In the section pictured in Figure 3 no inflammation, edema, fibrosis or hemorrhage was evident. Some kidneys from 25 week old control animals had occasional glomeruli undergoing atrophic degenerative changes.

Glomerular and tubular changes were observed in kidney sections from 25 week old animals fed HF (Figure 4, Figure 5). The tubules in the section in Figure 4 had swollen nuclei and epithelial cells. Tubular lumens contained proteinaceous debris. Tubular cells were swollen. Glomerular changes observed in this section were swelling and increased cellularity. Extensive glomerular changes in this group as seen in Figure 5 were infrequent.

Kidneys from GR animals at 35 weeks (Figure 6) had for the most part large glomeruli with open Bowman's spaces. There were some atrophic glomeruli and cystic tubules in these kidneys.

Glomerular changes of considerable magnitude were observed in kidneys of 35 week old animals fed the HF ration (Figure 7, Figure 10). These changes included hypertrophy of the capsular epithelium, swelling of some capillary loops and atrophy of others.

Tubular changes in the kidneys of 35 week old obese animals were extensive (Figure 8, Figure 9). These changes

included amorphous debris and proteinaceous material in the lumens, swelling of tubular epithelial cells and cystic tubules. These sections were negative when stained with Congo Red and Alcian Blue.

Glomerular crescents (Figure 10) were observed in kidneys from two experimental animals. These developed in response to some injurious stimuli.

A number of lesions were seen in the 45 week groups of animals fed GR and HF (Table 8) which were not observed in other groups. Kidneys from control animals at 45 weeks (Figure 11) had some degenerative changes in glomerular and tubular structure. For the most part, however, glomerular tufts were distinct with no hyperplasia or swelling. Bowman's space was narrow and open.

The 45 week old animals fed HF postweaning had kidneys with extensive damage (Table 9). This group showed considerably more degenerative changes and of greater magnitude than any other group (Table 7). An example of the extensive glomerular and tubular damage observed in these animals is illustrated in Figure 12. In this section tubules were dilated and contained proteinaceous secretion. Glomerular changes were also visible. A higher magnification of the tubular changes observed in most of the 45-week HF group is shown in Figure 12.

Special staining procedures were inconclusive. Sections as illustrated in Figure 12 were negative when stained with Congo Red, Alcian Blue and Oil Red O.

Figure 1. Kidney section from a weanling rat (078004). Numerous glomeruli are seen, inactive and active ones. Tubules are open and clear or may be collapsed as a result of inactivity. The brush border of the proximal convoluted tubules is incomplete due to immaturity. No inflammation or degenerative changes were seen in this kidney. Hematoxylin and Eosin, X160.

Figure 2. Kidney section from an animal 15 weeks old (078017) fed the high fat ration for 12 weeks. The tubules are functional, but some contain hypertrophied cells. An occasional epithelial cell has become detached and lies free in the tubule lumen. The most significant change is the glomerular degeneration as indicated by glomeruli A and B. Glomerulus A remains only as a dense atrophic mass while B is larger and has blood passing through the capillaries. The entire capillary tuft in B is smaller and is irregular compared to others. Hematoxylin and Eosin, X160.

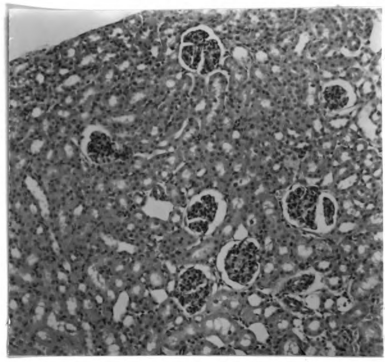

Figure 1

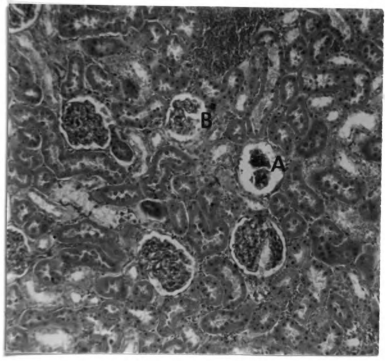

Figure 2

Figure 3. Kidney section from an animal 25 weeks old (078008) fed the grain ration after weaning. Glomeruli are large, well-defined and functional. Tubules are normal, open, and free of secretion or debris. No inflammation, edema, fibrosis or hemorrhage to indicate any disease process was found in this section. Occasional glomeruli were undergoing atrophic degenerative changes, probably as a result of the normal aging process. Hematoxylin and Eosin, X160.

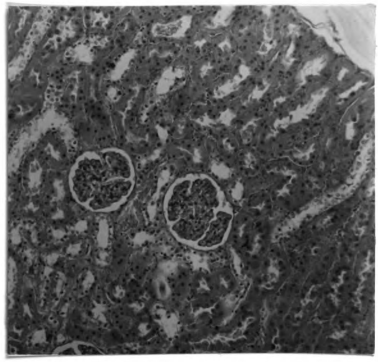

Figure 3

Figure 4. Kidney section from an animal (078010) 25 weeks old fed the high fat ration for 22 weeks showing glomerular and tubular changes. The most prominent change in this section is the swelling of the nuclei and epithelial cells of the tubules (arrow). The tubules contain proteinaceous debris (A) and epithelial cells are swollen. The glomerular tuft is swollen and is more cellular than normal (B). Hematoxylin and Eosin, X270.

Figure 5. Kidney section from an animal (078010) 25 weeks old fed the high fat ration for 22 weeks showing glomerular and tubular changes. The glomerular tuft in this photomicrograph remains only as an atrophic mass floating in secretion (arrow). The capsule is enlarged and Bowman's space contains secretion as a result of blockage below this level. Vacuoles indicate fat and the small dark globules are probably protein of serum origin, although they could be remnants of degenerating cells. The former is more likely. In this kidney section, occasional small lymphocytic foci are present but inflammation and general hemorrhage are not present with the exception of a small amount of edema and serous fluid collected around one arteriole. Hematoxylin and Eosin, X270.

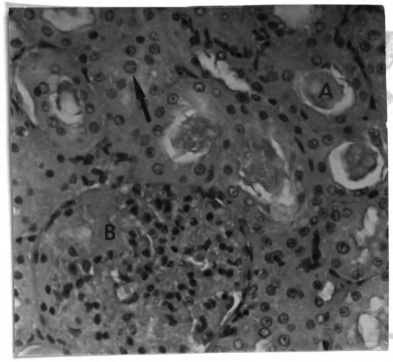

Figure 4

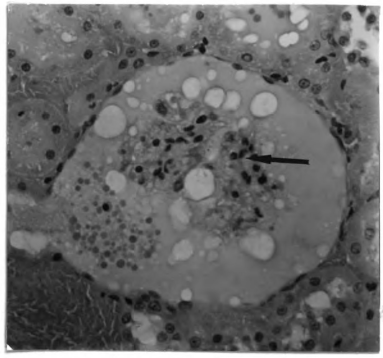

Figure 5

Figure 6. Kidney section from an animal (078024) 35 weeks old fed the grain ration for 32 weeks. Glomeruli appear normal. There is a fragment of capillary loop lying free in the glomerular space (arrow). Tubules appear open and normal. Hematoxylin and Eosin, X160.

Figure 7. Glomerular damage in kidney section from an animal (078026) 35 weeks old fed the high fat ration 32 weeks. Capillary loops are swollen so detail is obscured. Hypertrophy of capsular epithelium is visible in about half of the capsule surface (arrow). This has not increased to the point that it could be called an epithelial crescent. Other changes seen in this kidney included occasional cystic tubules with secretions or hyaline casts, somewhat shrunken glomeruli probably indicating early atrophic changes. There are a few collecting tubules in the medulla containing hyaline casts. These lesions were not extensive, however. Hematoxylin and Eosin, X270.

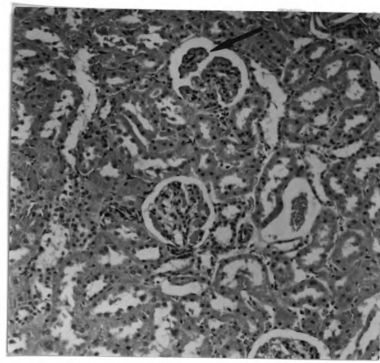

Figure 6

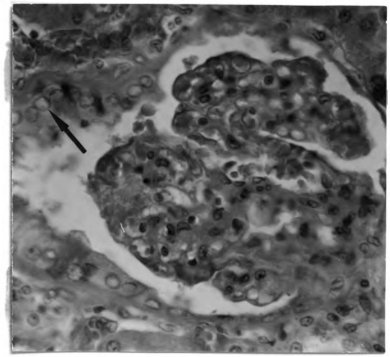

Figure 7

Figure 8. Tubular changes occurring in the kidney from a rat (078027) fed the high fat ration for 32 weeks. Changes include proteinaceous material in the lumens (arrow), swelling of tubular epithelial cells and amorphous debris in the tubules. There is a small focus of lymphocytes (A). Hematoxylin and Eosin, X270.

Figure 9. Additional tubular changes occurring in the same section as seen in the previous figure. A cystic tubule (A) and tubules containing secretion which appears to have coagulated (arrow) are evident. Smaller tubules contain debris in lesser quantities. This section was negative when stained using Congo Red and Alcian Blue. Hematoxylin and Eosin, X160.

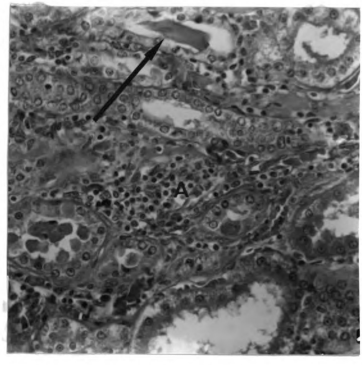

Figure 8

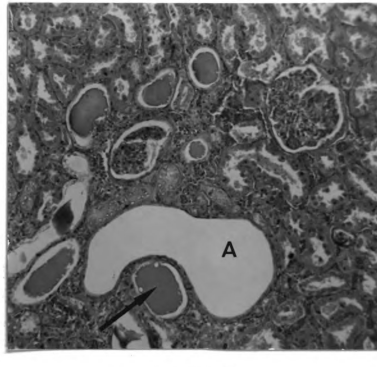

Figure 9

Figure 10. A glomerulus from the same section as seen in the two previous figures. Glomerular capillary and basement membrane detail are indistinct (arrow) although some loops are functional as indicated by the presence of erythrocytes in the lumens. A large epithelial crescent has formed at one area (A). The remainder of Bowman's capsule appears to be unaffected. Some material, which appears to be protein, is lying in Bowman's space. Hematoxylin and Eosin, X270.

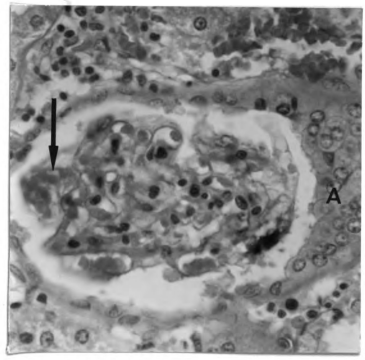

Figure 10

Figure 11. Kidney section from an animal (080119) 45 weeks old fed the grain ration for 42 weeks post-weaning. Glomerular tufts are distinct (A), capillary loops are well defined and Bowman's space is narrow and clear (narrow arrow). Bowman's membrane is normal (broad arrow). The broken capsule at the upper left surface is an artifact. Tubules are open and clear. Hematoxylin and Eosin, X160.

Figure 12. Glomerular and tubular changes in a section from an animal (080116) 45 weeks old fed the high fat ration for 42 weeks. Extreme degenerative changes are indicated by the large number of dilated tubules (A) and the obsolescence of glomeruli (arrows) in this section. Dilated tubules were distributed throughout this section extending from the surface throughout the cortex and into the medulla. Most of these tubules were widely dilated and some contained hyaline casts and secretion with protein. Many of the tubules were dilated, but empty. The epithelial cells were flattened and pressed close to the basement membrane. This could have been caused by the retained fluid which escaped when the sections were cut. In other areas the casts have remained and are visible in the dilated tubules. This section was negative when stained with Congo Red, Alcian Blue, and Oil Red O. Hematoxylin and Eosin, X60.

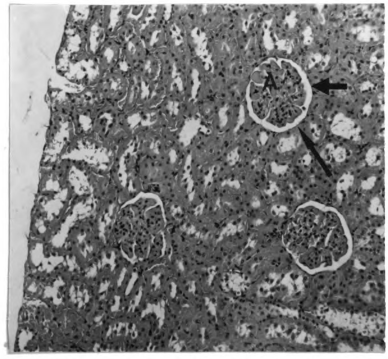

Figure 11

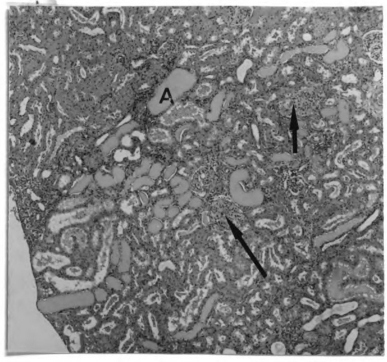

Figure 12

Figure 13. High-magnification view of tubular changes occurring in the same kidney section seen in the previous figure. The tubular lumens contain protein. Strands of fibrin attach the protein masses to the epithelial cell surfaces. Some epithelial cells have undergone degeneration and are no longer evident (arrow). Hematoxylin and Eosin, X270.

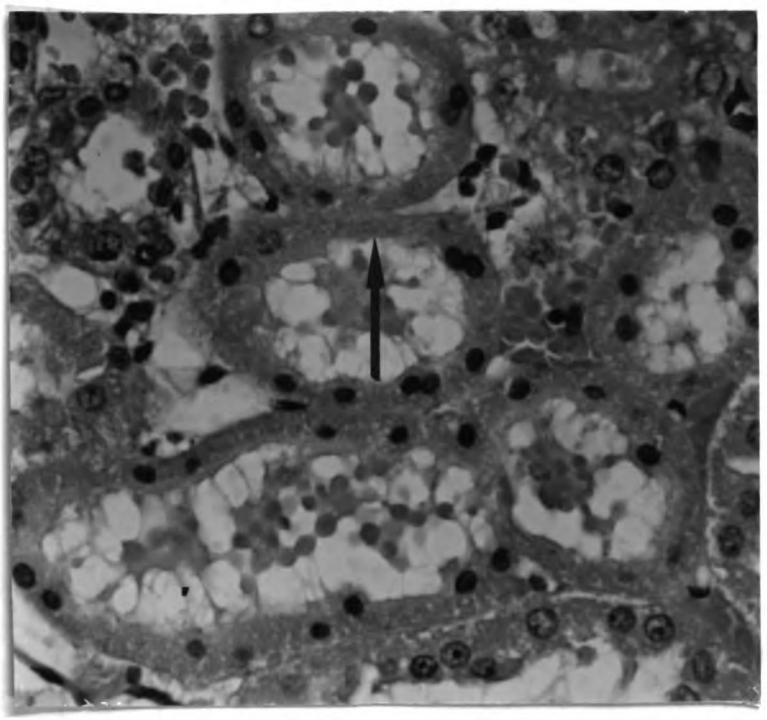

Figure 13

Table 3. Body weight, kidney weight and kidney weight/body weight ratio of Osborne-Mendel male rats fed GR or HF for varying periods of time.

Age[a]	Diet[b]	n	Body Weight[c]	Kidney Weight[d]			Total Kidney Weight to Body Weight Ratio (X100)
				Right	Left	Both	
Weanling	--	8	52.3± 3[e]	0.336±0.03	0.342±0.04	0.678±0.70	1.30 ±0.03
15 weeks	GR	5	388.8±21	1.431±0.05	1.373±0.05	2.804±0.09	0.726±0.02
	HF	5	456.6±22	1.500±0.06[fb]	1.370±0.05	2.870±0.11	0.630±0.01[fa]
25 weeks	GR	6	447.2± 9	1.575±0.04	1.526±0.03	3.101±0.06	0.694±0.01
	HF	6	641.5±23[fd]	1.821±0.04[fc]	1.703±0.07	3.524±0.10[fb]	0.552±0.02[fd]
35 weeks	GR	9	498.7±11	1.662±0.05	1.592±0.04	3.254±0.08	0.646±0.02
	HF	9	765.1±28[fd]	2.032±0.05[fb]	1.943±0.07	3.955±0.13[fc]	0.522±0.01[fc]
45 weeks	GR	9	510.1±12	1.722±0.07	1.682±0.05	3.402±0.12	0.667±0.01
	HF	9	779.6±23[fa]	2.338±0.10[fb]	2.250±0.13	4.588±0.23[fc]	0.589±0.02[fd]

[a]Age at sacrifice. All animals except weanling group had been fed diets for 3 weeks less than this age.

[b]The diets fed from weaning were the modified grain ration (GR) (Appendix) and the modified high fat ration (HF) (Appendix).

[c]Body weight refers to the live body weight and includes gastrointestinal contents.

[d]Kidney weights were recorded after the capsule was removed.

[e]Mean ± standard error.

[f]Statistically significant (Students two-sided paired "t" test--a=P<.05; b=P<.02; c=P<.01; d=P<.001) from control group of same age or in case of right kidney weights, significantly different from left kidney weight within the same group.

Table 4. Analysis of variance of body weight data.

	Source of Variation	df	SS	MS	F
	Age	4	668836	167209	80.27**
Error a	Pairs (Age)	26	54152	2083	
	Diet	1	648725	648725	172.17**
	Age X Diet	4	122342	30586	8.12**
Error b	Pair X Diet (age)	26	97964	3768	

** P<0.01

Table 5. Analysis of variance of kidney weight data.

	Source of Variation	df	SS	MS	F
	Age	4	13.56	3.39	3.29
Error a	Pairs (Age)	26	26.74	1.03	
	Diet	1	6.21	6.21	44.36**
	Age X Diet	4	2.46	0.62	4.43*
Error b	Pair X Diet (Age)	26	3.66	0.14	

** $P<0.01$

* $P<0.05$

Table 6. Analysis of variance of kidney weight/body weight ratio data.

	Source of Variation	df	SS	MS	F
	Age	4	0.3568	.0892	3.33*
Error a	Pairs (Age)	26	0.6969	.0268	
	Diet	1	0.1369	.1369	65.19**
	Age X Diet	4	0.0340	.0085	4.05*
Error b	Pair X Diet (Age)	26	0.05470	.0021	

** P<0.01

* P<0.05

Table 7. Percentage of animals in control (GR) and experimental (HF) groups with histopathologic kidney lesions.

Lesion or Change ↓	Group →	Weanling	15 weeks-GR	15 weeks-HF	25 weeks-GR	25 weeks-HF	35 weeks-GR	35 weeks-HF	45 weeks-GR	45 weeks-HF
	n →	8	5	5	5	4	9	9	9	9
Kidney Capsule										
Irregular			20							
Bowman's Capsule										
Thick					20		11			33
Fibrosis			20	20						22
Hyaline				20						
Crescent							11	11		33
Necrosis			40	80			11	22	11	67
Irregular					20		22			
Glomerular Tuft										
Atrophic (Immature?)		88	60	100	60	25	89	67	33	56
Adhesions			60		20	25	11	33		33
Fibrosis			20		20	50				
Degeneration					40					11
Bowman's Space										
Blood										
Protein			20		20		11	11		
Tubular Epithelium										
Hyperplasia										22
Degeneration								22		
Necrosis									11	
Cystic with Filtrate					20	25	56	44	100	90
Swelling			20			50				
Cellular Casts									22	
Fibrosis										11
Lymphocytes & Neutrophils									11	56
Basement Membrane Thickened										11
Henle's Loops										
Cell and Protein Casts			60	20	60		22	11	11	44
Hyaline Casts			20			25		11		
Hemoglobin Casts			20							
Corticomedullary Junction										
Cystic Tubules with Filtrate			20		20					
Protein Debris in Tubules					20					
Tubular Necrosis							22			
Collecting Tubules										
Casts								11		
Epithelium Degeneration		13						11		
Hemoglobin and Protein in Lumen		13		20						11
Renal Papillae										
Protein in Tubules				20	20					
Renal Pelvis										
Hemorrhage				20		25		11		
Miscellaneous										
Calcification			40	20	20					
Cortical Hemorrhage			20							
Nephritis										11
Focal Hemorrhage										11

Table 8. Lesions observed only in kidneys from animals 45 weeks old and fed the grain or high fat ration post-weaning.

Hyperplasia of Tubular Epithelium

Necrosis of Tubular Epithelium

Cellular Casts

Fibrosis

Lymphocytes and Neutrophils

Thickened Basement Membrane

Nephritis

Focal Hemorrhage

Table 9. Lesions observed only in kidneys from animals fed the high fat ration.

Casts in Collecting Tubules
Hyperplasia of Tubular Epithelium
Degeneration of Tubular Epithelium
Fibrosis of Tubular Epithelium
Thickened Basement Membrane
Hemoglobin and Protein in Collecting Tubule Lumens
Renal Pelvis Hemorrhage
Nephritis
Focal Hemorrhage
Hyalin in Bowman's Capsule

DISCUSSION

The Osborne-Mendel male rat when fed a high fat diet has a propensity for obesity (Mickelsen *et al.*, 1955; Schemmel, 1967; Schemmel *et al.*, 1969). Animals in this study fed the high fat diet (HF) were significantly heavier than controls fed a natural grain ration (GR) at 25, 35 and 45 weeks of age (Table 3). Grossly these animals contained more carcass fat, particularly in the perirenal depots. In most obese animals the kidneys were completely embedded in adipose tissue. No brown fat was observed in the renal area except in the weanling animals. Other researchers have reported that animals made obese by feeding high fat diets contain more carcass fat (Wierda, 1950; Mickelsen *et al.*, 1955; Schemmel, 1967; Schemmel *et al.*, 1969). The body weight was significantly influenced by age, diet and the interaction of these two factors (Table 3).

Arataki (1926) reported that the right kidney of female and male control rats was 2.3% and 2.1% heavier, respectively, than the left. Similarly, Wachtel *et al.* (1966) found that the ratio of the weight of the left kidney to the right kidney was always less than 1.0. In this study, however, there were no significant differences

in the two kidney weights in the GR animals at the ages studied (Table 3). If the differences in kidneys weights are analyzed on a percentage basis, the right kidney was at least 2% heavier than the left in the 15, 25, 35 and 45 weeks groups although these differences were not statistically significant ($P>0.05$; paired "t" test). The right kidney was significantly heavier than the left in HF animals at all groups postweaning. Whether this difference is physiologically significant is not known. One could speculate that the hemodynamics are altered in the obese animals and that these changes could lead to the differences observed. Blood flow measurements have not been made in these animals however.

The absolute weight of both kidneys was significantly greater in the HF rats at 25, 35 and 45 weeks of age (Table 3). These differences cannot be explained on a basis of the dietary nitrogen intake. The percent of protein in the HF and GR diets is approximately the same. The GR animals would normally consume more protein per day as their food intakes are greater on an absolute, but not caloric, basis. Thus the endogenous protein presented to the kidney is greater in the control animals. Some workers have suggested that renal enlargement in obese animals resulted from a nonspecific effect of hyperphagia and the obesity (Marshall *et al.*, 1957).

Other workers have reported that in rats absolute kidney weights increased linearly as body weight increased (Hatai, 1913; Webster *et al.*, 1947; Widdowson and McCance, 1960). Obese rats were not used in any of these studies, however, and it is doubtful that the relation of kidney weight to body weight is linear in excessively overweight animals.

The relative kidney weight or kidney weight/body weight ratio (KW/BW) ranged from 1.30 in the weanling animals to 0.522 in obese animals (Table 3). This ratio was less than that of controls in all HF groups. Other researchers have reported a KW/BW ratio greater than unity in young animals (Webster *et al.*, 1947; Widdowson and McCance, 1960). In these studies, this ratio decreased as age and body weight increased.

Marshall *et al.* (1957) found that kidney weights of animals made obese by several procedures (genetic, hypothalamic, GTG) were heavier than controls. The KW/BW ratio in these obese animals, however, was less than that of controls.

Grossly, the kidneys from HF and GR animals appeared normal. No pale, granular, pitted kidneys were observed in any of the animals. Similarly, none of the animals, HF or GR, had hydronephrosis, ureteral blockage or bladder stones.

Kidneys from the weanling animals can best be described as immature. The glomeruli and tubules appeared undifferentiated in the nephrogenic zone below the capsule (Figure 1). These kidneys were similar to those described in young animals by other workers (Bogomolova, 1966; Hirsch *et al.*, 1971). No evidence of inflammation or degenerative changes were seen in kidneys in this group.

The absolute incidence of renal lesions in the 15 and 25 weeks groups of control animals was greater than that of the obese animals (Table 7). The severity of the lesions, however, was greater in the HF groups.

By 35 weeks of age the obese animals had considerably more histological renal damage than control animals (Table 7). Glomerular and tubular lesions similar to those described as occurring spontaneously in rats with aging were observed (Saxton and Kimball, 1941; Simms and Berg, 1957; Andrew and Pruett, 1957; Kennedy, 1957; Bras and Ross, 1964; Gray and Purmalis, 1965). Cystic tubules with flattened epithelium (Figure 9; Figure 12) were particularly prevalent in kidneys from the obese animals. Glomerular damage leading to crescent formation (Figure 10) was similar to that reported by other researchers also (Gray, 1963; Bras and Ross, 1964).

The results reported may be complicated by the occurrence of an epidemic in the animal laboratory during

the course of this investigation. The etiologic agent isolated was *Pasteurella pneumotropica*. However, all animals except the weanlings were in the laboratory at the time of the epidemic. In spite of this complication, several conclusions can be made concerning the renal lesions in obese animals, especially as the animals aged.

SUMMARY

A histopathological examination of kidneys from animals at weaning and at 15, 25, 35 and 45 weeks of age, fed either a grain ration (GR) or a high fat (HF) ration postweaning, was completed.

The animals fed HF were significantly heavier than the GR controls after 25 weeks of age. These animals contained considerably more fat than the GR animals, especially in the perirenal-retroperitoneal depots. Age and diet each had a significant effect upon body weight. In addition, the interaction of diet and age on body weight was statistically significant.

In the HF-fed group, the right kidney was heavier than the left in animals at 15, 25, 35 and 45 weeks of age. There were no differences in the weights of the two kidneys in the GR groups at any age examined. The total kidney weight was significantly greater in the HF groups at 25, 35 and 45 weeks of age. The effect of diet on kidney weight was statistically significant as was the interaction of diet and age. The kidney weight to body weight ratio was significantly less in HF animals at all ages. Furthermore, this ratio was significantly influenced by the diet, age and the interaction of diet and age.

Lesions in kidneys from GR and HF animals affected the glomeruli and tubules. Prior to 35 weeks of age, the incidence of lesions in both groups was almost comparable, although the lesions in the kidneys from HF animals were more severe and covered more of the kidney parenchyma.

By 45 weeks of age and 42 weeks on the respective diets, the kidneys from the HF animals had considerably more damage than those from GR rats. The total number of lesions and the severity of these was greater in the HF group at this age.

In conclusion, dietary obesity had an adverse effect on the kidneys. A histopathological examination of the kidneys by light microscopy without specific staining for key enzymes may be a less sensitive tool for examining this effect of obesity on the kidneys than tests of functional capacity or examination by electron microscopy.

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PART III. Kidney function in dietary obesity: Effects of obesity and diet on renal transport of organic acids and bases.

INTRODUCTION

Renal tubular secretion, the transport of materials from peritubular fluid to tubular lumen is a process by which many substances are added to the urine. Substances actively transported across the tubular epithelium include certain organic acids and bases and hydrogen ions (H^+). Many compounds foreign to the body such as penicillin are transported by such a system, which is localized in and restricted to the proximal tubule (Wesson, 1969).

Organic ion secretory systems may be studied by at least three different approaches: (1) the intact organism may be used to study various agents and conditions that affect secretion, (2) the appearance and approximate concentration of visible dyes in the lumina of isolated tubules can be observed microscopically, and (3) an *in vitro* slice or isolated tubule technique using a suitable nutrient medium can be used (Wesson, 1969). The first method is limited to circumstances which are tolerated by the animal, and furthermore, extra-renal factors may affect the results. The visual method has the advantage that luminal accumulation of substrate can be distinguished from intracellular accumulation; however, quantitation is less precise and only colored or fluorescent compounds can be studied. The slice

technique permits the use of a wide range of environmental conditions (pH, temperature, ion concentration, etc.) and permits examination and precise quantitation of a great number of variables. Although the secretory system is incomplete in the *in vitro* preparation, the advantages of the technique are considerable.

For these reasons the *in vitro* slice technique developed by Cross and Taggart (1950) was used to elucidate the effect of obesity produced by feeding a high fat diet on tubular secretion in the male rat.

In vitro Measurement of Renal Transport

The technique developed by Cross and Taggart (1950) has been widely used to study the secretion of organic compounds. Very thin kidney cortical slices are incubated in an oxygenated buffered salt medium and the intracellular accumulation of compounds such as *p*-aminohippurate (PAH) is used as an indication of the activity and capacity of the secretory system. Accumulation of PAH and other organic compounds is expressed as the final ratio of concentrations in the slice and medium, or the slice/medium ratio (S/M ratio).

The *in vitro* methods used to study renal tubular transport have several advantages (Cross and Taggart, 1950) including the following: permit observation of events that accurately reflect tubular transport; can be used to simultaneously measure transport and certain

metabolic activities such as oxygen consumption; permit variation of experimental conditions (pH, ion concentration, temperature, etc.) over a broad range which would produce systemic toxicity in the intact animal; make it simple to rigidly control the chemical composition of the ambient fluid; and allow exclusion of extrarenal factors (hormones, blood flow, etc.) that may alter tubular secretion in the intact animal.

The *in vitro* slice preparation is a useful technique even though the secretory system is incomplete. The slice preparation does not allow for a continuous filtration process, with the resulting disturbance of normal concentration relationships between cells and tubular urine. Also, the PAH, once it has accumulated intracellularly in the *in vitro* technique, is able to diffuse out of the cortical slices back into the medium, in contrast to the unidirectional movement of PAH in the intact kidney. Another disadvantage of this system is the inability to distinguish between the accumulation of substrate within the cells from that accumulated within the tubular lumen. While these differences undoubtedly influence certain quantitative aspects of PAH transport, numerous experimental observations suggest that the same biochemical processes operate in both systems.

The slice technique is a reliable estimate of organic acid and base transport as shown by a number of

reports comparing transport *in vivo* and *in vitro*. In the rabbit *in vivo* studies have shown the maximum capacity of the renal cortex to concentrate PAH is 4-5 μmoles/g tissue, and similar values have been obtained using kidney slices (Foulkes and Miller, 1959a). Penicillin, PAH, or carinamide, competitive inhibitors of the acid transport system, have similar action, both *in vivo* and *in vitro*, on the intracellular accumulation of phenol red (Forster and Copenhaver, 1956). Other inhibitors such as dibenemine and dibenzyline depress N-methylnicotinamide (NMN) transport *in vitro* and *in vivo* (Ross *et al.*, 1968a). Mudge and Taggart (1950b) demonstrated that acetate and lactate have stimulatory effects on PAH transport in both preparations while succinate and fumarate uniformly depressed transport. Cross and Taggart (1950) and Mudge and Taggart (1950b) presented similar data supporting their conclusion that the accumulation of PAH by kidney slices *in vitro* and the tubular excretion of this compound in the intact animal are closely related phenomena.

A schemmatic representation of the data obtained from the slice incubation system is shown in Figure 14. The S/M ratio develops with time and reaches a plateau after 60-90 minutes. The system may be manipulated employing inhibitors or stimulators so that a wide range of S/M ratios may be obtained. A nitrogen atmosphere prevents active accumulation of PAH so that the S/M ratio approaches 1, indicating diffusion only.

The S/M ratio is the net result of intracellular accumulation, binding within the cell and extrusion out of the cell (runout). Intracellular accumulation involves an active transport process described below. The efflux mechanism, although incompletely characterized at this time, is distinctly different from the process mediating uptake (Farah *et al.*, 1963; Ross *et al.*, 1968b). Efflux, or runout, is thought to involve passive diffusion. There is also some evidence that an energy-requiring transport process may be associated with runout since inhibitors of PAH uptake such as dinitrophenol (DNP), octanoate, iodopyracet, and probenecid can decrease PAH runout (Kinter and Cline, 1961; Wilbrandt and Rosenberg, 1961).

Active transport refers to an energy-requiring process whereby a substance is transported across a biological membrane against an electro-chemical gradient. The energy required is obtained from cellular metabolism. Inhibition of a transport process by anoxia or by inhibitors of selective enzymes involved in the energy producing mechanism of the cell suggests that PAH transport is active. Low concentrations of azide, cyanide, and arsenite, exposure to cold and anaerobiosis all block PAH uptake (Wesson, 1969).

Theoretically, any active transport process has quantitative limits. In defining these limits it is

necessary to postulate the existence of some form of carrier system. The number of reactive sites on the carrier system is limited; that is, the system can only be transporting a limited number of molecules at a given moment. The active transport system for organic anions in the kidney exhibits a non-linear relationship between concentration and transport rate. The rate of transport increases as substrate concentration increases until saturation of the mechanism occurs. This fixed maximal transport rate suggests that active transport involves the reversible combination of the transported compound with a carrier system, which is limited. Also consistent with the concept of a cellular carrier of limited capacity is the demonstration of competitive phenomena associated with active transport systems such as penicillin competing with PAH for transport.

Active transport can also be limited by decreasing the energy available to the carrier system. Aerobic metabolism provides energy for the tubular secretory mechanisms for organic acids and bases. Tubular secretion is blocked by anoxia, by inhibitors of the cytochrome electron transport system, and of the oxidative reactions of the tricarboxylic acid cycle (Taggart and Forster, 1950; Shideman and Rene, 1951; Maxild and Mϕller, 1969). The energy-rich phosphates, such as adenosine triphosphate (ATP), generated by the oxidative

reactions apparently play a central role in providing energy for transport systems and may explain the dependence on aerobic metabolism. DNP, which uncouples oxidation and phosphorylation, depresses PAH transport *in vivo* and *in vitro* without affecting tubular reabsorption of glucose or amino acids. This strongly suggests that phosphate bond energy is involved in tubular secretory transport (Taggart, 1958).

Although the carrier system for organic acids has not been isolated and characterized, considerable evidence suggests that proteins are involved in organic ion transport. Ross *et al.* (1969) and Magour *et al.* (1969) used a receptor protection technique to provide evidence that the renal carrier of NMN is in the protein fraction. Similar techniques have not been utilized to identify the carrier for PAH since a specific, irreversible inhibitor of PAH transport is not yet available. The NMN studies were possible because Ross *et al.* (1968a) demonstrated that dibenamine specifically and irreversibly blocked NMN transport. That the transport system for PAH is protein is suggested by the work of Hirsch and Hook (1970c) who showed penicillin stimulation of PAH S/M ratios in young animals was associated with enhanced renal cortical protein synthesis.

Model of the Renal Secretory Mechanism

The secretory mechanism in mammalian renal cortical slices is thought to involve two distinct processes, the transport from peritubular fluid into the cell and the movement from the cell into the tubular lumen (Taggart, 1958). Movement into the cell requires active transport, whereas the step involving movement across the luminal border is downhill and involves diffusion only (Foulkes and Miller, 1959b, Tune *et al.*, 1969).

The multifaceted nature of the transport process was elucidated by two groups. Foulkes and Miller (1959b), using renal cortical slices, showed that there are two intracellular fractions of PAH, a fraction which rapidly diffuses and equilibrates with extracellular PAH and a fraction which is slowly equilibrating and is responsible for the high slice to medium ratio. Foulkes (1963) and Welch and Bush (1970) also presented evidence for at least two intracellular pools--a bound or compartmentalized fraction and a freely diffusible pool.

On the basis of these results, Foulkes and Miller (1959b) proposed a 4 step mechanism of renal tubular secretion of PAH (Figure 15). The proposed steps are as follows: step one consists of diffusion of PAH from the medium to the interstitial fluid; step two involves facilitated diffusion at the peritubular cell membrane from the interstitial fluid into the cell; step three

consists of an active accumulation of a high tissue concentration of PAH; and step four results in the transfer of PAH across the luminal border of the cell into the tubular lumen.

Since the S/M ratio is the net result of uptake into the slice, accumulation in the cells and runout back into the medium, uptake may involve the first three steps and runout may involve the fourth. Ross *et al.* (1968b) presented data suggesting that the uptake of organic acids and bases occurs at the peritubular side and that runout occurs at the luminal side of the proximal tubular cell.

Sheikh and Møller (1970) reported that, in contrast to earlier views, active transport of PAH ascribed to the rapid component and slow component may be due to intracellular compartmentalization. These workers used separated renal tubules and eliminated the extracellular space and thus the possibility that the slow component of PAH transport might be due to a diffusion barrier between the exterior and interior parts of the slices. Both of these occur in the slice technique.

Recently, Tune *et al.* (1969), using isolated tubules, proposed a model similar to that one of Foulkes and Miller (1959b). These workers propose that entry into the tubular fluid occurs down a concentration gradient by a process consistent with simple diffusion.

Furthermore, active transport into renal tubular cells across the peritubular membrane with subsequent diffusion down a concentration gradient into the tubular lumen follows. Tissue PAH levels reach a steady state when passive efflux equals the active influx (Tune *et al.*, 1969).

Factors Affecting Renal Tubular Transport of Organic Acids and Bases

A number of factors affect the renal tubular transport of organic acids and bases *in vitro* and *in vivo*.

Metabolites

Intermediary metabolites may act to stimulate or inhibit the organic acid transport system. For instance, acetate enhances PAH transport in man (McDonald *et al.*, 1951), dog (Mudge and Taggart, 1950b), and rabbit (Cross and Taggart, 1950). Lactate and pyruvate, precursors of acetate, also increase PAH uptake by renal kidney slices *in vitro* (Cross and Taggart, 1950) and PAH transport *in vivo* in man (McDonald *et al.*, 1951). Low concentrations of dicarboxylic acids of the citric acid cycle, as succinate and fumarate (Cross and Taggart, 1950), fatty acids of intermediate carbon chain length (C_6-C_{10}) (Cross and Taggart, 1950; Schacter *et al.*, 1955) and the amino acids L-alanine and L-glutamate (Schacter *et al.*, 1955; Cross and Taggart, 1950), markedly depress the uptake of

PAH by renal cortical slices. The maximal tubular excretory capacity of PAH (Tm_{PAH}) is depressed in the dog by succinate (Mudge and Taggart, 1950b), fumarate (Mudge and Taggart, 1950b) and α-ketoglutarate (Knoefel and Huang, 1959). Balagura-Baruch and Stone (1969) demonstrated that in the dog PAH transport was depressed by α-ketoglutarate when plasma levels of PAH were both greater than and less than the Tm_{PAH}. This depression was not overcome by the addition of acetate. These workers concluded that α-ketoglutarate is a noncompetitive or mixed inhibitor of the PAH transport system. Farah *et al.* (1963) showed that acetate and lactate decreased while succinate and α-ketoglutarate increased the rate of runout of PAH from renal slices.

Metablic Inhibitors

Metabolic inhibitors also depress transport of organic acids and bases. Taggart and co-workers (Cross and Taggart, 1950; Mudge and Taggart, 1950a; and Taggart and Forster, 1950) have shown *in vitro* and *in vivo* that 2,4-dinitrophenol, cyanide, and arsenite depress PAH, phenolsulfonthalein (PSP) and Diodrast uptake. They proposed that these compounds depress organic acid transport by acting directly on some component of the cellular transport mechanism, probably by inhibiting aerobic phosphorylation. Maxild and Møller (1969) demonstrated that inhibiting the Krebs cycle and

carbohydrate metabolism virtually abolishes PAH accumulation. Metabolic inhibitors may also depress organic acid S/M ratios by increasing runout (Kinter and Cline, 1961; Farah *et al.*, 1963). As with acids, organic base transport is inhibited by metabolic inhibitors (Shideman and Rene, 1951; Farah *et al.*, 1959).

Electrolyte Composition

The electrolyte composition of the bathing medium and of the slices influences organic acid and base transport.

The H^+ concentration of the medium may affect the transport system, and the optimum pH for maximum accumulation is species specific (Copenhaver and Davis, 1965; Ross *et al.*, 1968b; Hirsch, 1970).

Potassium is necessary for the transport system to function. Taggart *et al.* (1953) found kidney slices lost the ability to accumulate PAH in a K^+-free medium and suggested K^+ was necessary for maintenance of cell integrity. Foulkes and Miller (1960) proposed that K^+ has a specific role at the cell membrane. They found that adding K^+ to K^+-deficient slices stimulated PAH uptake prior to a significant increase in intracellular K^+. Furthermore, they suggested that K^+ is required as a part of the intracellular PAH-concentrating mechanism. Hirsch (1970) found that renal cortical slices from

immature and adult rabbits required K^+ in order to accumulate PAH. Burg and Orloff (1962a) reported that the digitalis glycoside, strophanthidin, interferes with PAH accumulation in slices of rabbit renal cortex, presumably by decreasing the K^+ content. When K^+ was increased in the medium, PAH accumulation was not depressed by strophanthidin. Dantzler (1969) similarly reported that increased K^+ in the medium overcame the depressing effects of ouabain, a cardiotonic steroid, on PAH accumulation. Ross *et al.* (1968b) demonstrated that decreased K^+ in the medium depressed uptake of PAH and NMN by dog renal slices without affecting runout. Chung *et al.* (1970) reported a critical role for Na^+ and Ca^{++} in addition to a requirement for K^+ for accumulation of PAH and PSP by rabbit kidney slices. Dantzler (1969) reported that snake and chicken kidney slices were unable to accumulate PAH and urate normally in the absence of K^+. If K^+ was sufficient, transport of PAH was not affected unless Na^+ was greatly reduced. Thus a number of electrolytes have been reported to influence organic acid and base transport.

Compounds Transported by the Same System

Other compounds transported by the same system may affect the transport of PAH or NMN in the *in vitro* slice preparation. Chlorothiazide (Beyer and Baer, 1961) probenecid (Kinter and Cline, 1961; Huang and Lin, 1965),

DNP (Huang and Lin, 1965), free fatty acids of medium chain length (Schacter *et al.*, 1955), Diodrast (Cross and Taggart, 1950), penicillin (Cross and Taggart, 1950), carinamide (Cross and Taggart, 1950), PSP (Forster and Copenhaver, 1956), triiodothyronine (T_3) and tetraiodothyronine (T_4 or thyroxine) (Nepomuceno and Little, 1964a; 1964b; Hirsch, 1970), have all been shown to depress PAH accumulation.

The PAH transport system in the immature animal may mature or develop at a faster rate if one of these compounds which inhibits *in vitro* is given *in utero* or shortly after birth. T_3 given to weanling rats increased the ability of renal cortical slices to transport PAH and increased the kidney weight (Hirsch and Hook, 1969b). Since T_3 when added to slices *in vitro* depressed PAH uptake these workers suggested that the compound is transported by the organic acid system and specifically stimulates the transport system. Folic acid when administered to rats several days prior to sacrifice also stimulated PAH accumulation by renal cortical slices (Hirsch and Hook, 1969c).

Treating nursing rats or pregnant rabbits with penicillin increased PAH accumulation when measured *in vitro* (Hirsch and Hook, 1969a, 1970a, 1970b), presumably by increasing the synthesis of specific transport protein(s) (Hirsch and Hook, 1970c). NMN transport was

unaffected in treated animals, thus the substrates of the organic acid transport system were apparently specific stimulators of the PAH system.

Farah *et al.* (1959) reported that transport of NMN by the organic base system in renal slices from dogs was depressed by tetraethylammonium (TEA) and darstine, both bases and both presumably transported by the same system. Ross *et al.* (1968a) found dibenamine and dibenzyline depressed NMN transport *in vivo* and *in vitro* 80-90% without affecting organic acid transport as measured by PAH accumulation. TEA uptake was inhibited by triiodothyropropionic acid and triiodothyroacetic acid, according to Nepomuceno and Little (1964b). Domer (1960) force fed NMN to dogs at 12-hour intervals for seven days and determined that renal tubular transport of NMN and priscoline (tolazoline) *in vivo* was increased.

Numerous studies have shown that other compounds transported by the same system may interfere with organic acid and base transport.

Structure

Compounds transported by the organic acid transport system may have a variety of chemical structures (Taggart, 1958). There may be intramolecular reactive groups possessed in common (Despopoulous, 1965). Weiner *et al.* (1964) point out that protein binding, acidic strength, lipid solubility, urinary pH, urinary flow, and

plasma level all may affect the transport of organic acids in a particular species. They also state that all acid compounds transported are transported as anions.

Effects of Uremic and Normal Serum

A number of workers have demonstrated that addition of "uremic" serum to the incubation system depressed PAH transport (White, 1966; Preuss *et al.*, 1966; Bourke *et al.*, 1967; Hook and Munro, 1968; and Orringer *et al.*, 1971). Hook and Munro (1968) found that fasting rats for 24 hours prior to removing the kidneys for the *in vitro* study also depressed PAH transport without affecting NMN transport. Adding acetate to the slice increased PAH S/M ratios to control values. Serum from nephrectomized animals also depressed PAH uptake but did not change NMN transport.

Orringer *et al.* (1971) found when normal serum was used at high concentrations, accumulation of PAH by rat renal cortical slices was depressed while at low concentrations it was stimulated. Azotemic serum, at any concentration studied, depressed transport (Orringer *et al.*, 1971). They suggested that, in high enough concentration, some constituent in normal serum competitively inhibited organic anion transport. Preuss *et al.* (1966) found that dialyzing uremic serum prior to adding it to the *in vitro* slice preparation prevented the inhibition.

Uninephrectomy in rats increased hippurate transport by the remaining kidney transiently while no change was seen in organic base transport (Goldberg *et al.*, 1970). These workers suggested the early increase in organic acid transport in hypertrophying kidneys is secondary to substrate induction in the growing kidney.

The Effect of Age and Developmental Stage of the Animal

Barrows and co-workers (Adams and Barrows, 1963; Beauchene *et al.*, 1965) reported depressed transport of PAH and α-aminoisobutyric acid (AIB) in kidney cortical slices from old rats. Associated with this depressed transport was a 10% decrease in deoxyribonucleic acid (DNA) and depressed sodium-potassium-activated adenosine triphosphatase (ATPase) activity in the older animals when compared with the younger ones.

Kidney cortical slices from the newborn of a number of species are incapable of accumulating as much organic acid and base as those from a mature animal (Williamson and Hiatt, 1947; New *et al.*, 1959; Rennick *et al.*, 1961; Hirsch, 1970).

The depth of the cortex from which the slices are taken may also affect the accumulation. The inner slices of renal cortex are more developed at birth than the outer slices of the "nephrogenic zone" (Wachstein and Bradshaw, 1965). In addition, the state of development of the animal at birth influences the transport system.

The guinea pig, which can take solid food at birth, has a well developed transport system at birth while the renal tubular transport system in the newborn rabbit is less mature (Hirsch, 1970).

The Tm_{PAH} was 2-4 times greater in premature infants fed a high protein diet for four weeks than in those fed a low protein diet, suggesting to Calcagno and Lowe (1963) that diet could induce renal tubular maturation. No changes were observed in glomerular filtration rate (GFR) or renal plasma flow (RPF) in these infants, so that the organic acid transport system was apparently selectively stimulated.

Sex

Most workers find that sex differences affect the accumulation of PAH by renal cortical slices. Renal cortical slices from mature male rats had greater PAH uptake than did those from females (Huang and McIntosh, 1955; Ferguson and Matthews, 1963). These workers demonstrated that the sex differences in the PAH S/M ratio is due to the stimulatory effect of testosterone and not to the inhibitory effect of estrogen. Farah *et al.* (1956) observed no difference between males and females in ability of kidney slices to accumulate PAH. Kleinman *et al.* (1966), however, reported that transport of PAH *in vivo* and *in vitro* was higher in males than females. Bowman (1970) also reported increased PAH transport in

renal cortical slices from male rats. S/M ratios for NMN and TEA, however, were the same for male and female rats. Apparently the increased S/M ratios obtained with kidneys from male rats is a result of increased uptake and decreased runout of PAH (Bowman, 1970).

Effects of Manipulations of Transport System on O_2 Consumption by Renal Cortical Slices

Copenhaver and Davis (1965) found no significant changes in oxygen consumption when the pH of the media ranged from 6.5 to 8.5. Taggart *et al.* (1953) reported similar respiration values for slices developing S/M ratios from 3.8 to 12.6. Although strophanthidin interferred with PAH transport, presumably by affecting the K^+ concentration in the medium, no concomitant change in oxygen consumption was observed (Burg and Orloff, 1962b). Bourke *et al.* (1967) found nephrectomized serum depressed PAH transport in kidney slices, but did not affect slice oxygen consumption. Chung *et al.* (1970) did not find changes in oxygen consumption with changes in PAH and PSP transport produced by using Na^+, K^+, and Ca^{++} in the medium in varying concentrations.

Huang and McIntosh (1955) reported a marked decrease in PAH uptake and oxygen consumption in kidney slices from hyposectomized rats, but suggested these were secondary to changes in testosterone and estrogen as a result of the hypophysectomy.

New *et al.* (1959) reported that oxygen consumption based on the nitrogen content of slices was lowest in the newborn, the age group which also accumulated the least PAH. These workers suggested that perhaps oxygen consumption and PAH transport were related.

The purpose of this study was to demonstrate that renal transport capacity is different in animals made obese by feeding a high fat diet. The *in vitro* slice preparation (Cross and Taggart, 1950) was used since it excludes factors that may alter tubular secretion in the intact animal. The specific objectives of this study were (1) to demonstrate the effect of obesity on the renal secretion of the organic acid, PAH and (2) to determine factors affecting the accumulation of PAH by renal cortical slices from these animals.

Figure 14. Schematic diagram of the *p*-aminohippurate (PAH) slice incubation system under several conditions. The PAH S/M ratio is equal to the concentration of PAH in the slice to that in the medium.

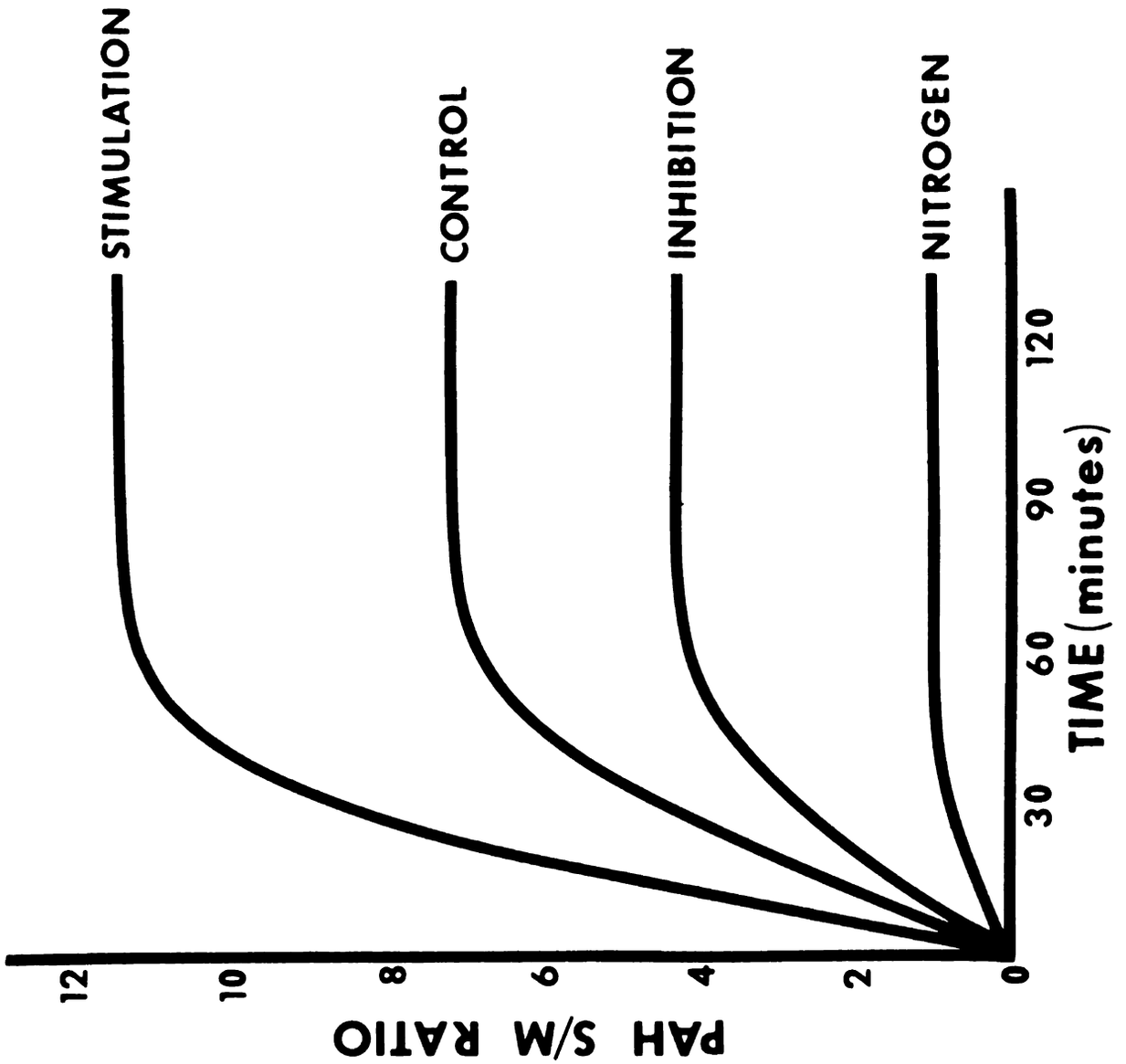

STIMULATION
CONTROL
INHIBITION
NITROGEN
PAH S/M RATIO
12
10
8
6
4
2
0
30
60
90
120
TIME (minutes)

Figure 14

Figure 15. Model of the PAH transport mechanism in the renal cortical slice as proposed by Foulkes and Miller (1959b). The following abbreviations are used: ISF--interstitial fluid, ICF--intracellular fluid, M--PAH in the medium, E--PAH in the interstitial fluid, pah--rapidly diffusible fraction of PAH, and PAH--slowly equilibrating intracellular fraction of PAH resulting from the active concentrating mechanism of Step 3.

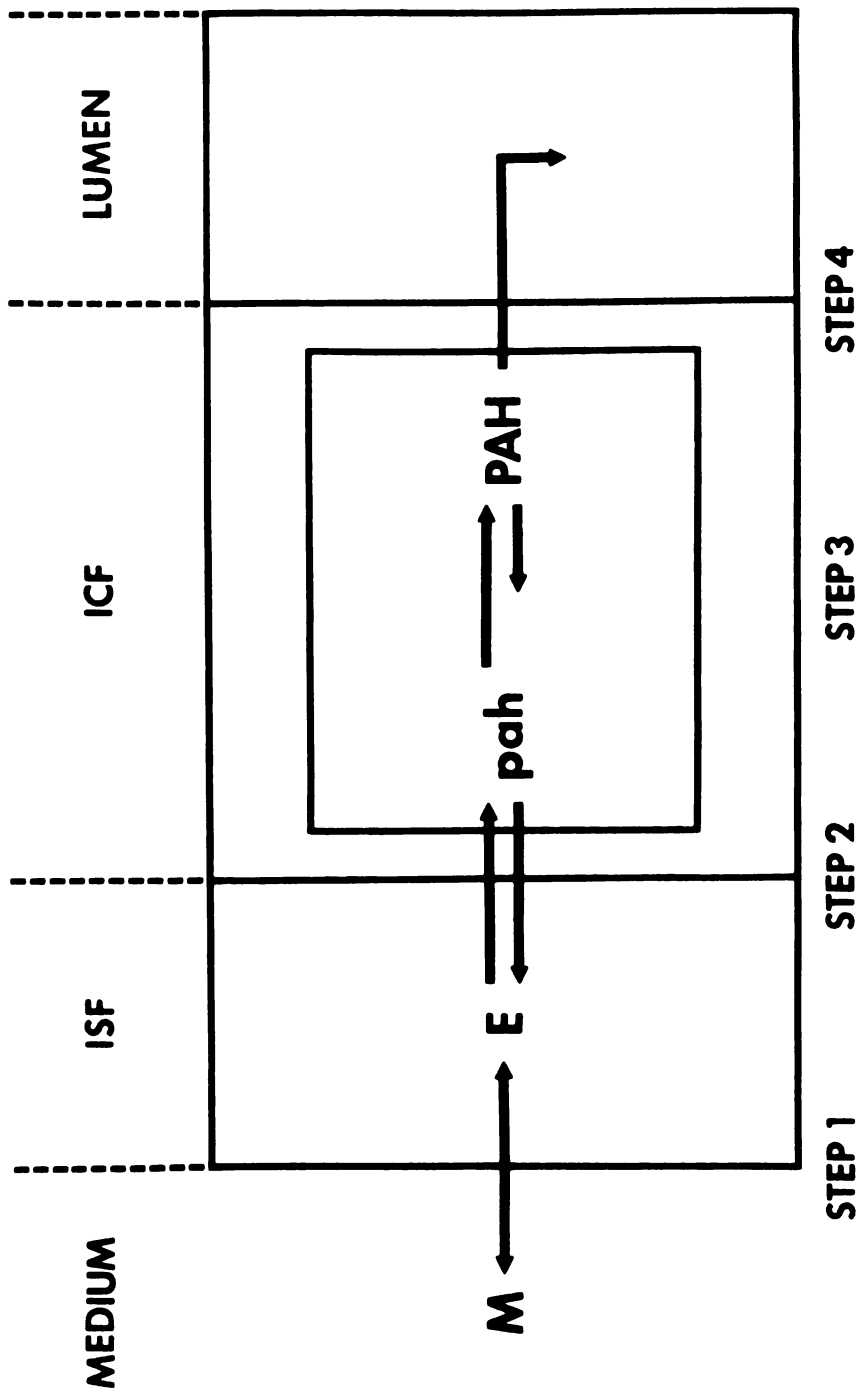


Figure 15

METHODS

Accumulation of Organic Ions by Renal Cortical Slices

The Cross and Taggart (1950) slice technique was used to study the ability of renal cortical slices to actively accumulate organic ions. PAH was used as the prototype to study organic anion transport while NMN was used as the prototype for studying organic cation transport.

Osborne-Mendel male rats of NIH stock and bred in the Human Nutrition Laboratory were fed either a grain ration (GR) (Appendix A; Campbell *et al.*, 1966) or a 60% fat ration (HF) (Appendix B; Mickelsen *et al.*, 1955) which has been shown to produce obesity in this strain (Schemmel *et al.*, 1969). The animals were housed in a temperature controlled room with a 12-12 hour light-dark cycle. Food and tap water were available *ad libitum*.

Animals were killed by cervical dislocation or by guillotine after obtaining the body weight. The kidneys were rapidly removed, trimmed of fat and capsule, and placed in iced normal saline. Kidney weights were obtained and renal cortical slices of 0.3-0.4 mm thickness were prepared free hand. Approximately 100 mg of tissue was added to the incubation media. The latter was a phosphate buffer media as devised by Cross and Taggart (1950) containing 7.4×10^{-5} M PAH and 6.0×10^{-6} M NMN-C^{14} (4.6 mc/mmol) and adjusted to pH 7.4.

Incubations were carried out in a Dubnoff Metabolic Shaker at 25°C under a gas phase of 100% oxygen. Incubations were for a 90-minute period except during initial uptake experiments. After incubation, slices were quickly removed from the media, blotted, and weighed. A 2 ml aliquot of the medium was taken from each beaker. Three ml of 10% trichloroacetic acid (TCA) were added to graduated cylinders containing tissue or medium. The tissue was macerated with a glass stirring rod. Slice and medium samples were brought to a final volume of 10 ml with distilled water and centrifuged at 1400 rpm for 10 minutes. After centrifuging, 2.5 ml of the supernatant were used to determine PAH spectrophotometrically as described by Smith *et al.* (1945). When NMN-C^{14} was used to study base transport, 1 ml of slice or media supernatant was added to scintillation vials containing 10 ml of modified Bray's solution (6 g of 2,5-diphenyloxazole and 100 g of napthalene per liter of dioxane). Radioactivity was determined using a Beckman LS-100 liquid scintillation counter, employing external standardization. All samples were counted to an accuracy level of ±2.00%.

Transport was expressed as the slice to medium (S/M) ratio which is equal to the concentration of PAH per gram of tissue (wet weight) divided by the concentration of PAH per ml of media or in the case of NMN-C^{14},

disintegrations per minute per gram of tissue (wet weight) divided by the disintegrations per minute per ml of medium.

The Effect of Serum on Accumulation of PAH by Renal Cortical Slices

To study the effect of serum on the accumulation of PAH by renal cortical slices, 0.5 or 1.0 ml of serum was added to 2.5 ml of medium prior to incubation. Blood was obtained from animals at the time of sacrifice. This was allowed to clot and the serum was harvested after centrifugation. S/M ratios obtained with the addition of serum to the media were compared with those obtained when saline in the same volume as serum was added. Kidneys from control animals (GR) were incubated in serum from GR- and from HF-fed rats. Serum from HF-fed rats were used with renal cortical slices from GR- and HF-fed rats.

Kinetic Analysis of PAH Uptake

Slices were incubated for 2 and 12 minutes at PAH concentrations of 2,4 and 8 x 10^{-4} M in order to estimate the rate of PAH transport. PAH was estimated spectrophotometrically as previously described. Uptake was calculated as the amount of PAH accumulated per gram of slice per minute between 2 and 12 minutes incubation. A Lineweaver-Burk plot (Clark, 1964) was used to plot the results. The reciprocal of the rate of PAH uptake per

minute was plotted against the reciprocal of the PAH concentration.

Oxygen Consumption of Renal Cortical Slices

Oxygen consumption of slices was determined using a multiple-unit constant pressure microrespirometer developed by Reineke (1961). Reaction vessels were incubated in a 37° water bath and shaken at 60-100 cycles/minute. Pure oxygen was used to gas all reaction vessels. After 30 minutes equilibration, the system was closed and oxygen consumption recorded at 15 minute intervals for 60-75 minutes. The kidney slices were prepared from rats that were killed by a sharp blow on the head. Kidneys were rapidly removed and placed in iced Ringer's-Phosphate (R-P) solution. Cortical slices 0.4-0.5 mm thick were cut with a Stadie-Riggs microtome and placed in R-P solution. Slices were blotted and approximately 100 mg weighed and placed in each reaction vessel in a R-P-200 mg % glucose solution. Triplicate samples were used for O_2 consumption studies. In addition triplicate samples from each kidney were blotted, weighed, and dried to constant weight at 95°C to determine dry matter content. These samples were then ether-extracted using a Goldfisch apparatus to determine fat content.

Oxygen consumption was calculated on wet weight (w) and dry weight (d).

QO_{2_W} and QO_{2_D} were used to express the µl of O_2/mg wet or dry tissue/hour respectively. All values were corrected to standard temperature and pressure (STP).

Renal Cortical Slice Composition

Slices were prepared as described previously for accumulation studies and kept in iced saline until blotted, weighed and placed in a drying oven for 24 hours at 100°C. Total water content of cortical slices was determined as the difference between the wet and dry weights and tissue water was expressed as a percent of the wet weight.

After drying, the total ether-extractable fat content of the renal cortical slices was determined for the dried sample using the Goldfisch apparatus. In some cases, it was necessary to pool dried tissue samples for fat determinations. The content of fat was expressed on a wet or dry basis as mg per 100 mg of wet or dry tissue weight, respectively.

Plasma Free Fatty Acids

Blood samples from nonfasted animals were obtained by heart puncture from animals fed the GR or HF ration for 4 5 weeks postweaning. These were stored for free fatty acid determinations at a later time by the colorimetric method of MacKenzie *et al.* (1967).

Histological Study of Renal Cortical Slices

Slices were prepared as described previously for accumulation studies. Some slices were used in a standard 90-minute accumulation study. In addition, several slices from the same beaker were placed in Zenker's solution (Armed Forces Institute of Pathology, 1968) (50 g potassium dichromate, 70 g mercuric chloride, and water to 2000 ml) for 10-12 hours, washed overnight in cold running tap water to remove excess mercury salts and placed in 70% ethanol in coded vials. A double-blind study was made to determine if histological differences between renal cortical slices from GR and HF-fed rats could be demonstrated. Kidneys were imbedded in paraffin and sections 2 μ thick prepared. Hemotoxylin and eosin and PAS stains were used (Armed Forces Institute of Pathology, 1968; Lillie, 1965).

Statistical Analyses

All data were analyzed statistically using Student's "t" test, paired or group comparison unless otherwise noted (Steel and Torrie, 1960). For those studies involving the effect of different PAH concentrations on the velocity of uptake, a kinetic analysis employing a Lineweaver-Burk plot (Clark, 1964) was used. Linear regression analysis (method of least squares, Goldstein, 1964) was used in determining the effect of age, body weight, and kidney weight on PAH and NMN accumulation and

the effect of body weight on kidney weight. In all statistical tests, the 0.05 level of probability was used as the criterion of significance.

RESULTS

Accumulation of the Organic Acid, PAH, by Renal Cortical Slices

Organic acid transport by renal cortical slices was depressed in HF animals (Figure 16). When kidney cortical slices from these animals, at different ages, were incubated for 90 minutes in the Cross and Taggart medium, a PAH S/M ratio of 4.81±0.29 (mean ± S.E.) developed (Figure 16). Animals of comparable ages fed the GR diet had kidneys which developed PAH S/M ratios of 6.96±0.41 under the same incubation conditions (Figure 16). These values are significantly different from those obtained from HF animals ($P<0.001$).

Factors Affecting the Accumulation of PAH by Renal Cortical Slices

The ability of renal cortical slices to accumulate PAH was inversely correlated with age (Figure 17), body weight (Figure 18), and kidney weight (Figure 19). As either of these variables increased, the transport of PAH decreased. This was true for both groups of rats fed either HF or GR. The inverse relationships were significant ($P<.01$) with GR, HF, or all data pooled.

Kidney weight was positively correlated with body weight (Figure 20), independently of the diet ($P<.01$).

Rate of Initial Uptake of PAH

The rate of PAH uptake by renal cortical slices from 12-week old GR rats was greater than that of slices from HF rats of the same age (Figure 21). The results are plotted on a double reciprocal plot (Lineweaver-Burk plot) and are expressed in Figure 21. The slopes of lines for GR and HF animals were 0.391 and 0.360 respectively. The plot for the GR group exhibited a V_{Max} of 39.97 (µg/g/min) while the V_{Max} for the HF group was 14.92 (Table 13). Km values for GR and HF animals were 15.62 and 5.37 (10^{-4} moles/L) respectively (Table 13).

The rate of PAH uptake by renal cortical slices from 60-week old GR rats was greater than that for slices from HF rats of the same age (Figure 22). The results are again plotted on a double reciprocal plot. The slopes of the lines for GR and HF animals were 0.490 and 0.318 respectively. The plot for the GR group exhibited a V_{Max} of 12.45 (µg/g/min) while the V_{Max} for the HF group was 4.63 (Table 13). Km values for GR and HF animals were 6.10 and 1.47 (10^{-4} moles/L), respectively (Table 13).

Accumulation of the Organic Base, NMN, by Renal Cortical Slices

To study the specificity of the inhibition of the anionic transport system, the accumulation of the organic base, NMN, was determined. In some cases, simultaneous accumulation of PAH and NMN was measured.

The accumulation of NMN by renal cortical slices from HF animals was no different from that of GR animals (Figure 23). Slices from animals fed GR incubated under the conditions as described previously (90 minutes, Cross and Taggart medium, 100% oxygen) developed NMN S/M ratios of 6.75±0.33 (Figure 23). This was not significantly different ($P>.05$) from the NMN S/M ratios (6.55±0.40) obtained when slices from HF animals were incubated similarly.

Factors Affecting the Accumulation of NMN by Renal Cortical Slices

The accumulation of NMN by renal cortical slices was significantly correlated with age ($P<0.05$) (Figure 24) and kidney weight ($P<0.01$) (Figure 25) in the HF group, but not in the GR group. When all data were pooled regardless of diet, NMN accumulation was significantly correlated with age ($P<.05$) (Figure 24) and kidney weight ($P<.01$) (Figure 25). NMN accumulation was not correlated with body weight (Figure 26) for any group ($P>.05$).

Effect of Changing Diets on Accumulation of Organic Acid and Base

Since the previous results suggested that accumulation of PAH by renal cortical slices was depressed in animals fed HF when compared to those on GR, the effect of the diet *per se* was investigated. A summary of the effect of diet switching on PAH accumulation by kidney cortical

slices and its comparison with the standard dietary regimens are given in Table 10. Animals fed HF for any period of time had depressed accumulation of PAH when compared to animals fed GR for any period of time (Figure 16, Figure 27, Table 10). Animals fed GR and switched to HF at least two days prior to sacrificing had renal cortical slices which accumulated PAH as if the animals had been on the HF ration throughout the experiment (Figure 27, Table 10). The accumulation of PAH by renal cortical slices from HF animals was significantly less than that by slices from GR animals (Figure 16, Figure 27).

When animals were fed HF and switched to GR at least two days prior to being sacrificed, kidney slices from these animals developed PAH S/M ratios not significantly different ($P>.05$) from those obtained with kidneys from animals fed GR continuously (Figure 16, Figure 27). The accumulation of PAH by kidney slices from animals fed GR or HF and switched to GR was significantly greater than that by kidney cortical slices from animals fed HF or GR and switched to HF (Figure 16, Figure 27, Table 10).

NMN accumulation by renal cortical slices was unaffected by diet switching (Figure 28, Table 11). The effect of diet switching on the accumulation of organic base by renal cortical slices as measured by NMN S/M ratios is summarized in Table 11. When animals were fed GR and switched to HF, accumulation of NMN was not

significantly different (P>.05) from that by renal cortical slices from animals fed HF and switched to GR (Figure 28, Table 11).

Effect of Serum on Accumulation of PAH by Renal Cortical Slices

Since the previous results indicated that the depressed accumulation of PAH by renal cortical slices was due in large part to the diet, the effect of serum on accumulation of PAH was determined. To test this, kidney slices from animals fed GR or HF were incubated with the normal media plus serum from GR or HF animals (Table 12). Accumulation of PAH by renal cortical slices incubated with media plus saline was used as a control against dilution effects.

In all cases, the accumulation of PAH by renal cortical slices was enhanced by the addition of serum independent of the source of serum or kidneys (Table 12). Accumulation was unaffected by the addition of an equal volume of saline (Table 12). The PAH S/M ratios developed by renal cortical slices from HF or GR animals incubated in HF serum were not different (P>0.05). Those developed by renal cortical slices from HF or GR animals with the addition of GR serum were not different (P>0.05). Furthermore, there were no differences in the PAH S/M ratios developed when HF serum was used when compared with GR serum (P>0.05).

The S/M ratios obtained with all serum-kidney combinations were significantly greater ($P<0.05$) than those with the dilution control (2.5 ml medium + 0.5 ml saline) or with the control medium (2.7 ml). There were no significant differences in the PAH S/M ratios obtained in 2.5 ml medium plus 0.5 ml saline when compared with 2.7 ml medium ($P>0.05$).

Histological Study of Renal Cortical Slices

The histological sections revealed kidneys in both groups that were normal for rats of the age used (30 weeks old) (Figure 29, Figure 30). No apparent morphological differences were distinguishable between kidney slices from HF and GR animals in histological sections by light microscopy (Figure 29, Figure 30). In both groups, tubules were normal except for slight dilation. Infrequent casts were present in kidneys from both groups. No evidence of kidney infection or inflammation was seen. Glomerular changes observed in kidneys from both dietary groups included focal hypercellularity, increased mesangial tissue, and limited metaplasia.

Kinetic Analysis of PAH Uptake

The rate of PAH uptake by renal cortical slices from 12 and 60 week old animals on GR and HF was determined.

PAH uptake by renal cortical slices from HF animals was less than that from GR animals independent of age

(Figure 21, Figure 22, Table 13). Kidneys from young animals on either diet had greater uptakes than those of older animals (Figure 21, Figure 22, Table 13). A summary of the kinetic analysis is given in Table 13.

Oxygen Consumption by Renal Cortical Slices

The oxygen consumption by renal cortical slices from kidneys of rats fed GR and HF is given in Table 16. There were no significant differences in the oxygen consumption on a dry or a wet weight basis ($P>0.05$).

Renal Cortical Slice Composition

The fat and water composition of renal cortical slices from kidneys of rats fed GR and HF is given in Table 17. There were no significant differences in the percent of water and total ether-extractable fat of the slices from the two groups ($P>0.05$).

Figure 16. Accumulation of PAH (S/M ratio) in renal cortical slices from male rats of different ages fed the control grain ration (GR) or the experimental high fat ration (HF). Each bar represents the mean ± (S.E.) obtained from duplicate determinations on the number of animals shown in parentheses. The value obtained from the HF group is significantly less than that of the GR group ($P<0.001$).

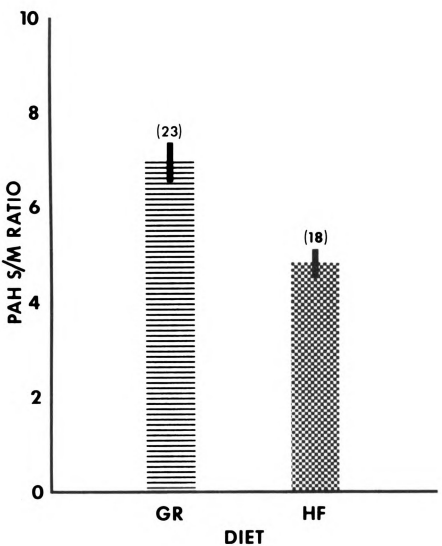


Figure 16

Figure 17. Accumulation of PAH (S/M ratio) in renal cortical slices from GR (□) and HF (○) rats plotted against age. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the average of duplicate determinations for individual rats. All three lines demonstrate significant regression ($P<0.01$).

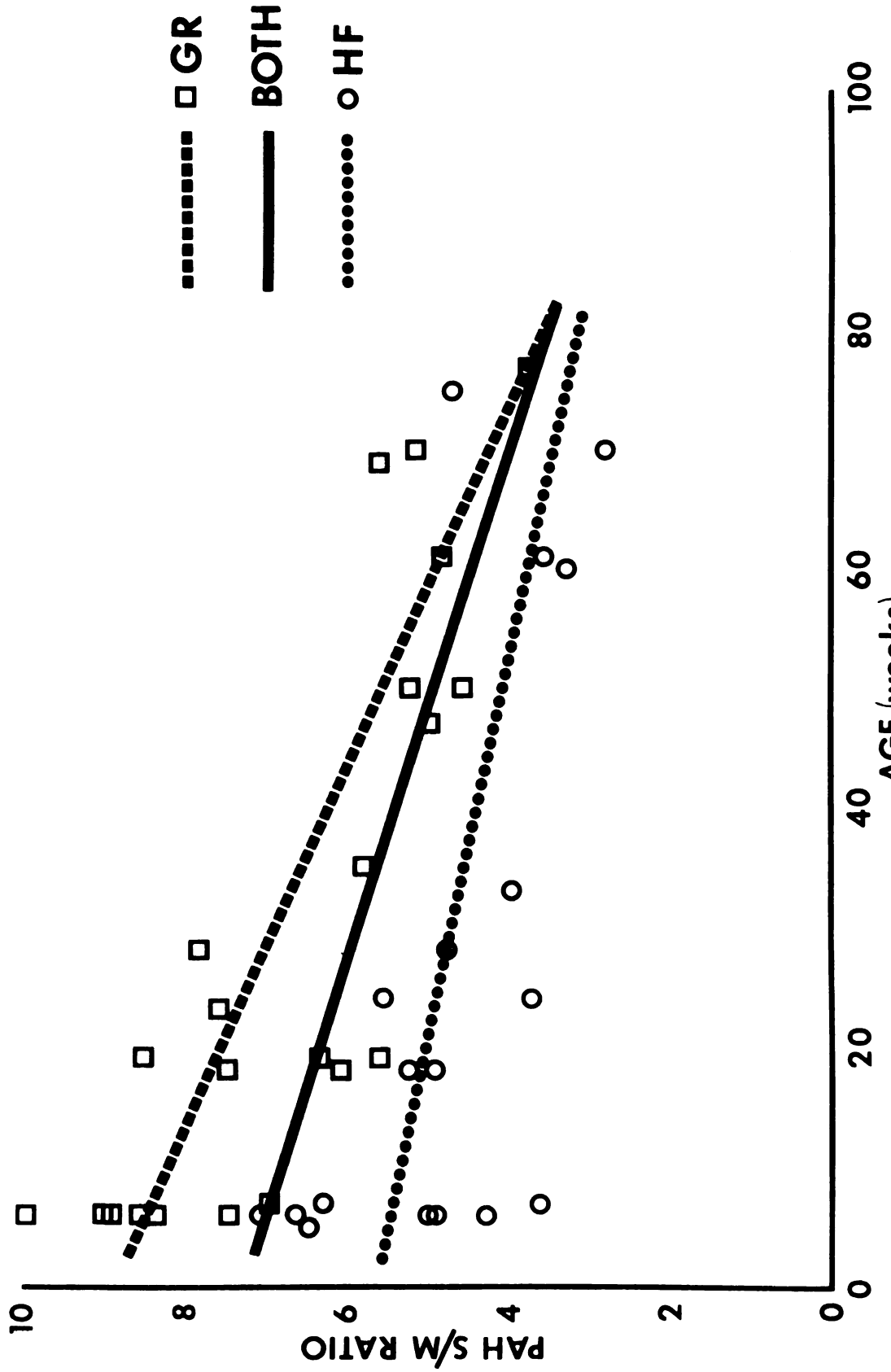


Figure 17

Figure 18. Accumulation of PAH (S/M ratio) in renal cortical slices from GR (□) and HF (○) rats plotted against body weight. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the average of duplicate determinations for individual rats. All three lines demonstrate significant regression ($P<0.01$).

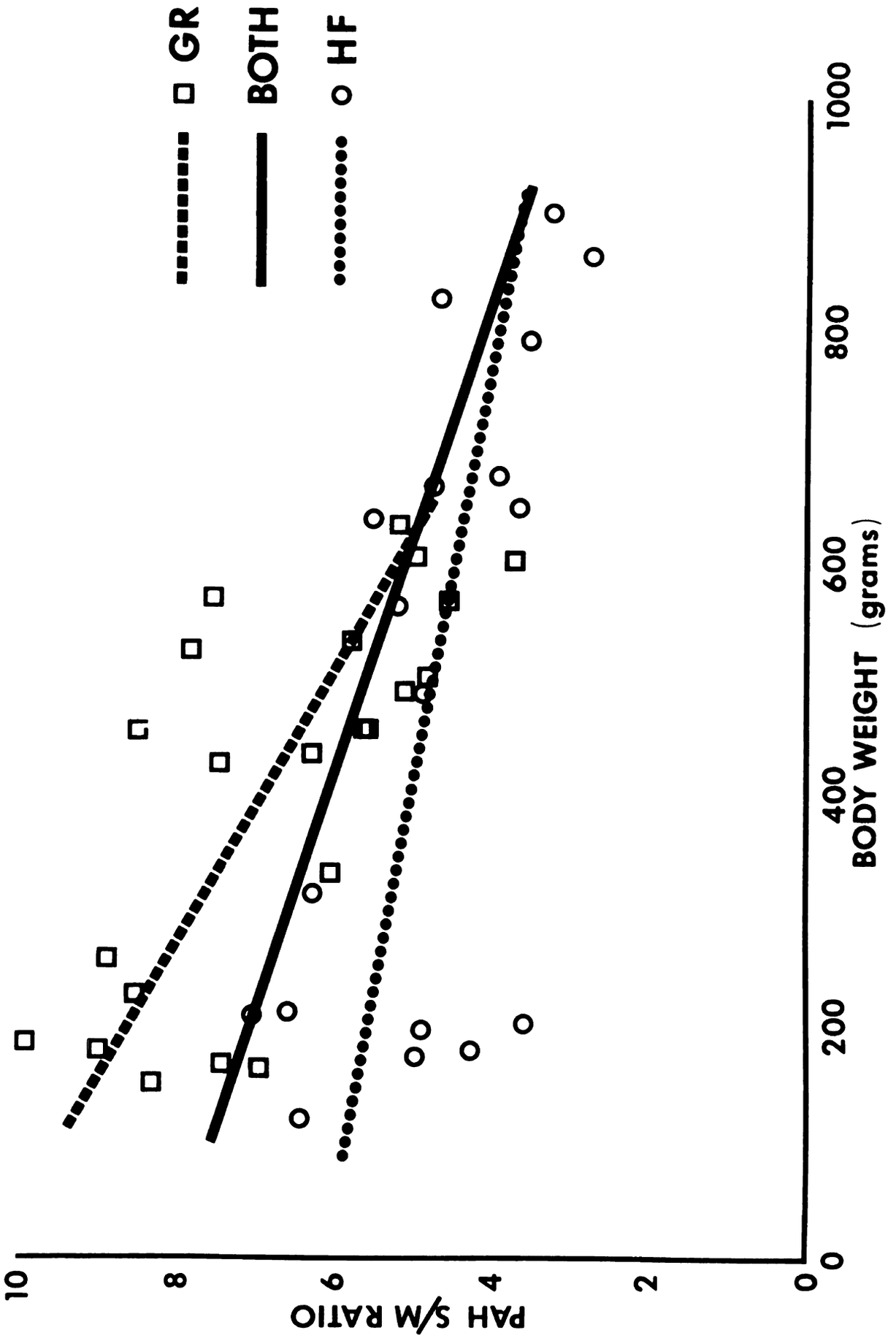

GR
BOTH
HF
PAH S/M RATIO
BODY WEIGHT (grams)
0
2
4
6
8
10
0
200
400
600
800
1000

Figure 18

Figure 19. Accumulation of PAH (S/M ratio) in renal cortical slices from GR (□) and HF (○) rats plotted against kidney weight. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the average of duplicate determinations for individual rats. All three lines demonstrate significant regression ($P<0.01$).

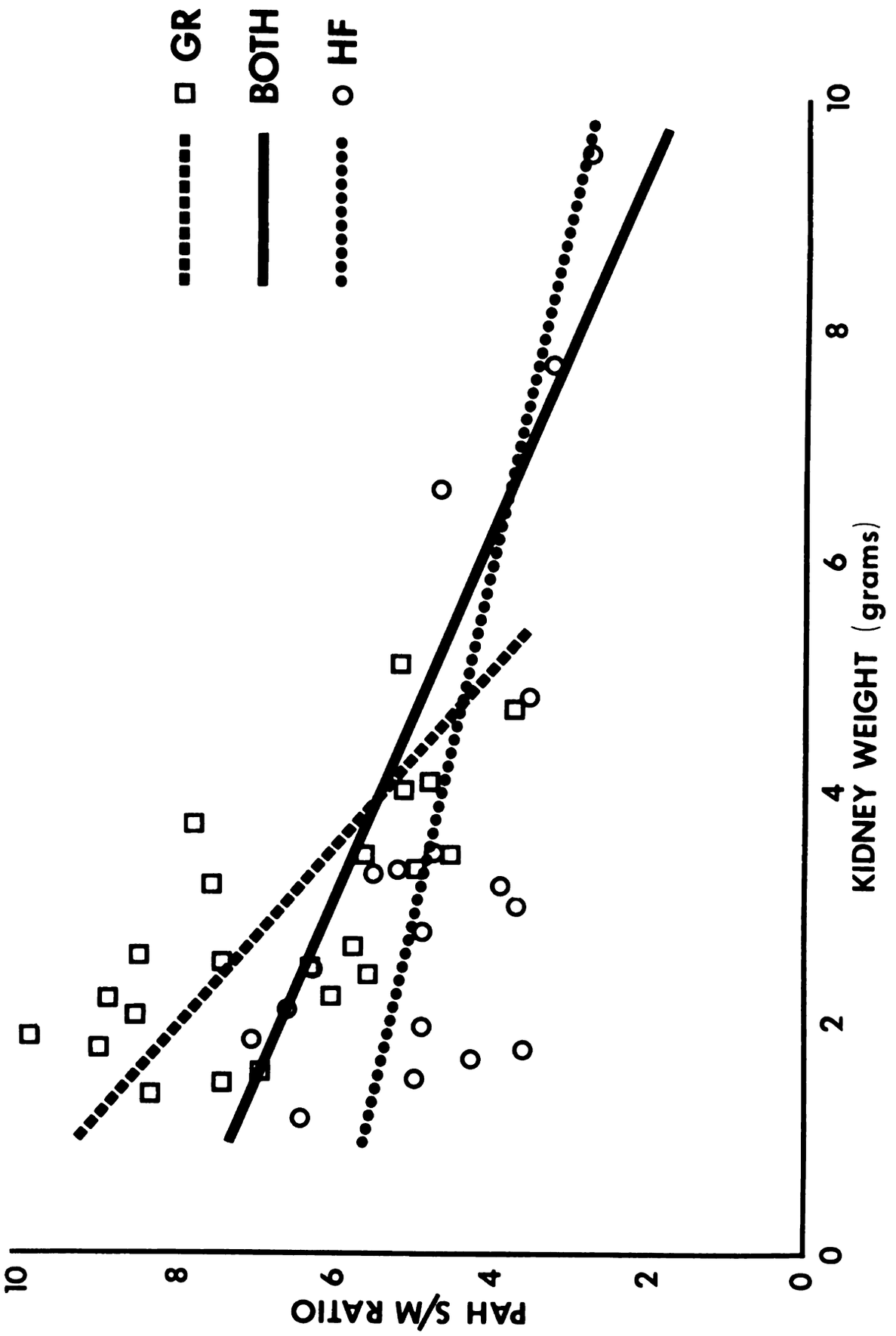


Figure 19

Figure 20. Effect of body weight on kidney weight in GR (□) and HF (○) rats. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the weights of both kidneys from individual rats. All three lines demonstrate significant regression ($P<0.01$).

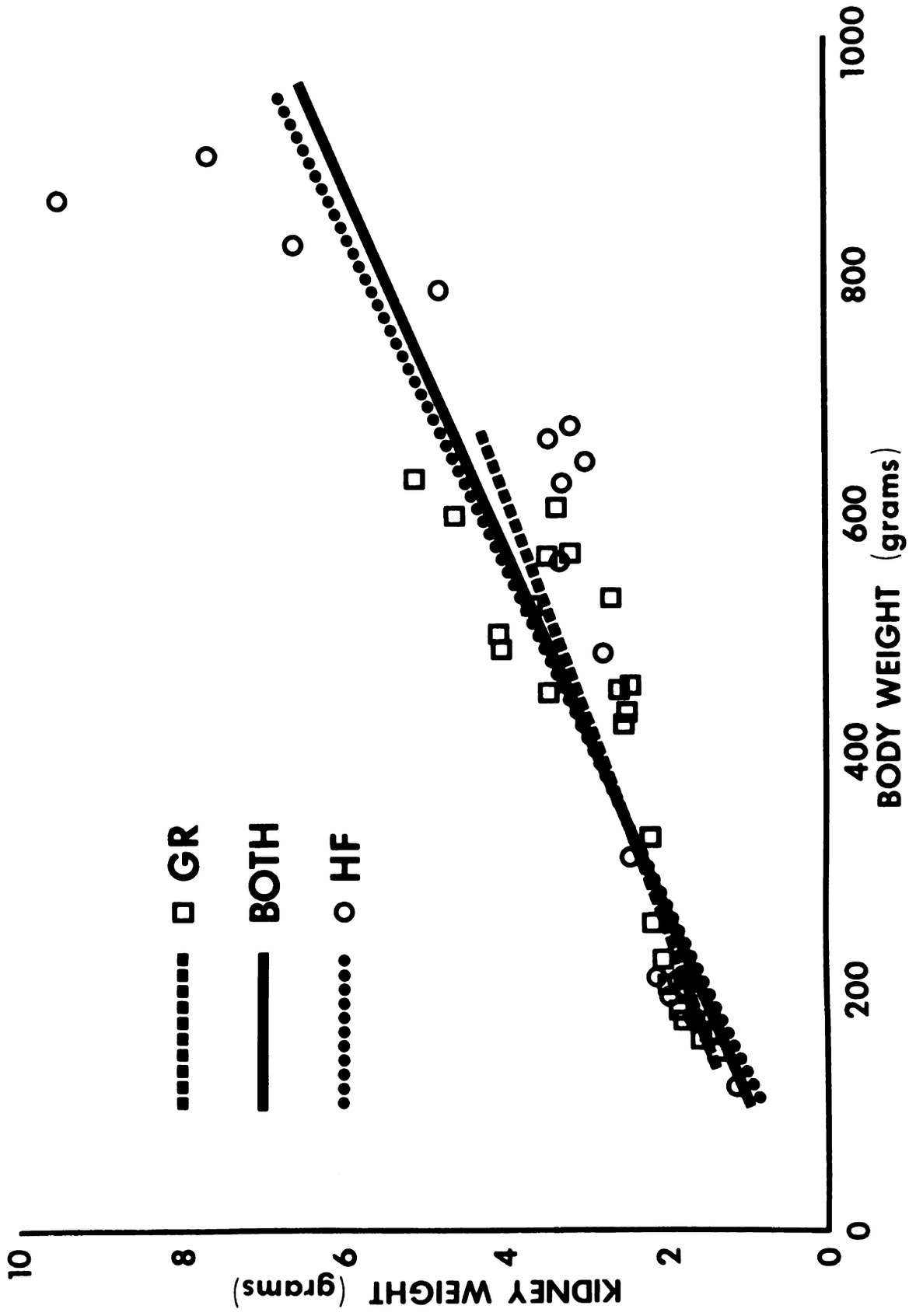

KIDNEY WEIGHT (grams)
BODY WEIGHT (grams)
GR
BOTH
HF
0
2
4
6
8
10
200
400
600
800
1000

Figure 20

Figure 21. Kinetic analysis of PAH uptake by renal cortical slices from 12 week old rats fed GR (□) or HF (○) using the Lineweaver-Burk plot. The rate of PAH uptake (µg/g/min) at PAH concentrations of 2, 4, and 8 x 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 minutes. The points indicate the means from 3 experiments. The slopes of the curves are 0.360 (HF) and 0.391 (GR).

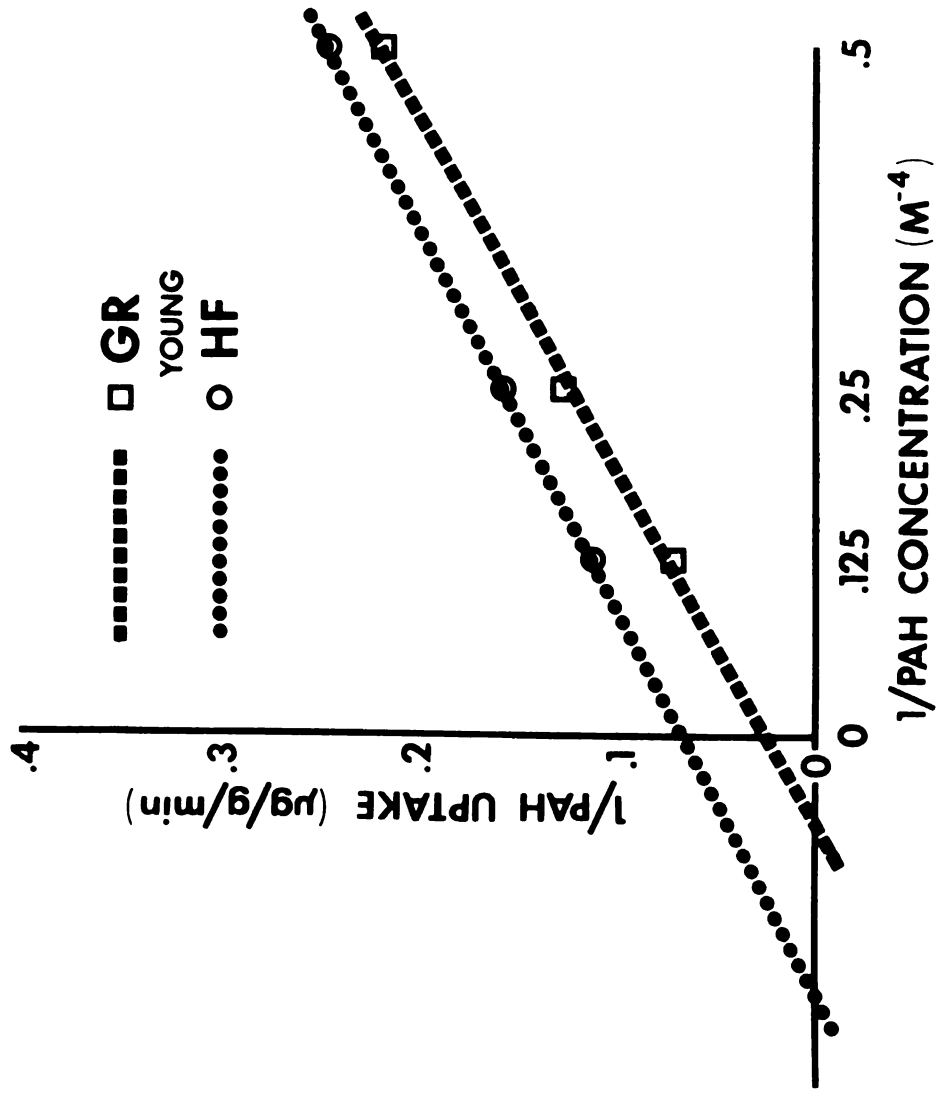

GR
YOUNG
HF
1/PAH UPTAKE (μg/g/min)
.4
.3
.2
.1
0
0
.125
.25
.5
1/PAH CONCENTRATION (M^{-4})

Figure 21

Figure 22. Kinetic analysis of PAH uptake by renal cortical slices from 60 week old rats fed GR (□) or HF (○) using a Lineweaver-Burk plot. The rate of PAH uptake (μg/g/min) at PAH concentrations of 2, 4, and 8×10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 minutes. The points indicate the means from 3 experiments. The lines are calculated regression lines. The slopes of the curves are 0.490 (GR) and 0.318 (HF).

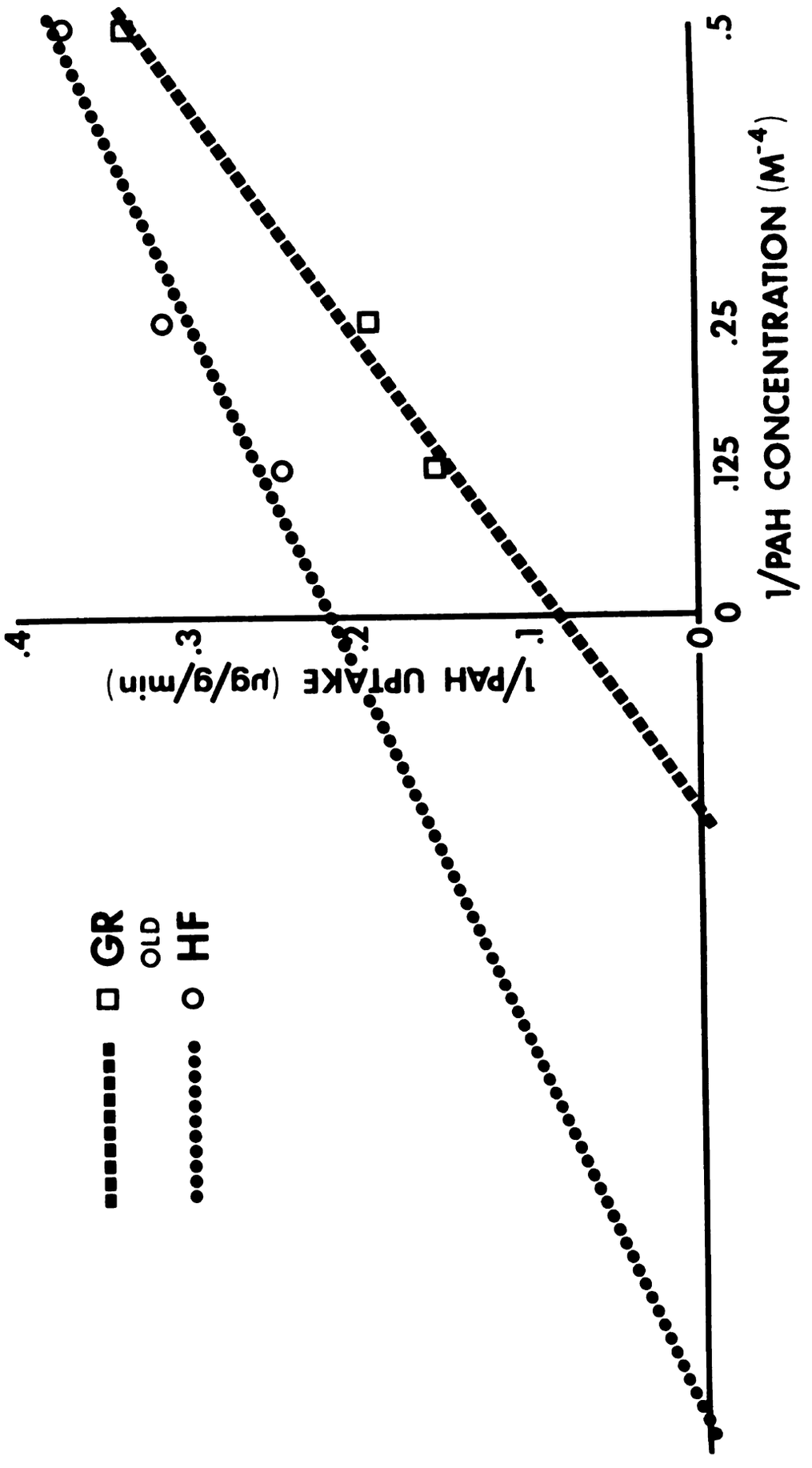

GR
OLD
HF
1/PAH UPTAKE (μg/g/min)
.4
.3
.2
.1
0
0
.125
.25
.5
1/PAH CONCENTRATION (M^{-4})

Figure 22

Figure 23. Accumulation of NMN (S/M ratio) in renal cortical slices from male rats of different ages fed GR or HF. Each bar represents the mean ± (S.E.) obtained with duplicate determinations on the number of animals in parentheses. The values obtained are not significantly different (P>0.05).

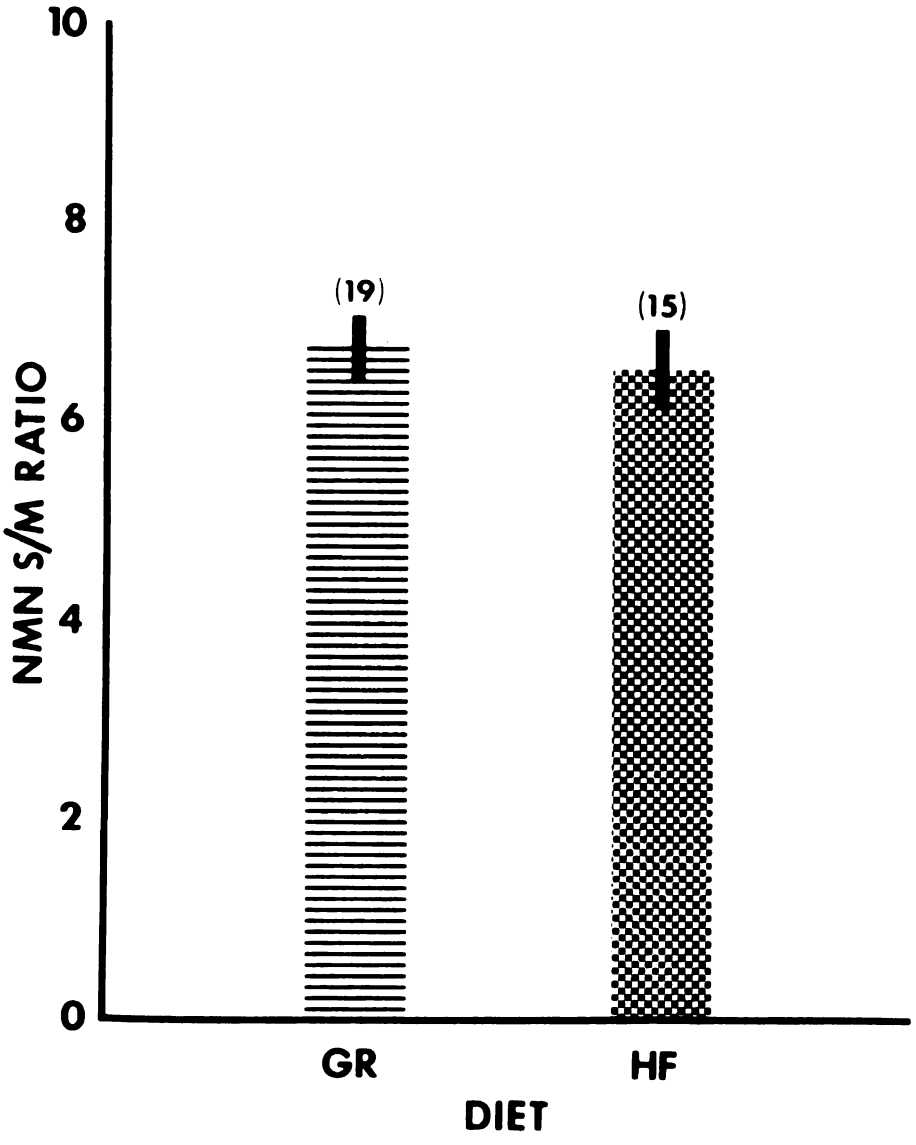


Figure 23

Figure 24. Accumulation of NMN (S/M ratio) in renal cortical slices from GR (□) and HF (○) rats plotted against age. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the average of duplicate determinations for individual rats. NMN accumulation in GR animals is not correlated with age ($P>0.05$). The other two lines demonstrate significant regression ($P<0.05$).

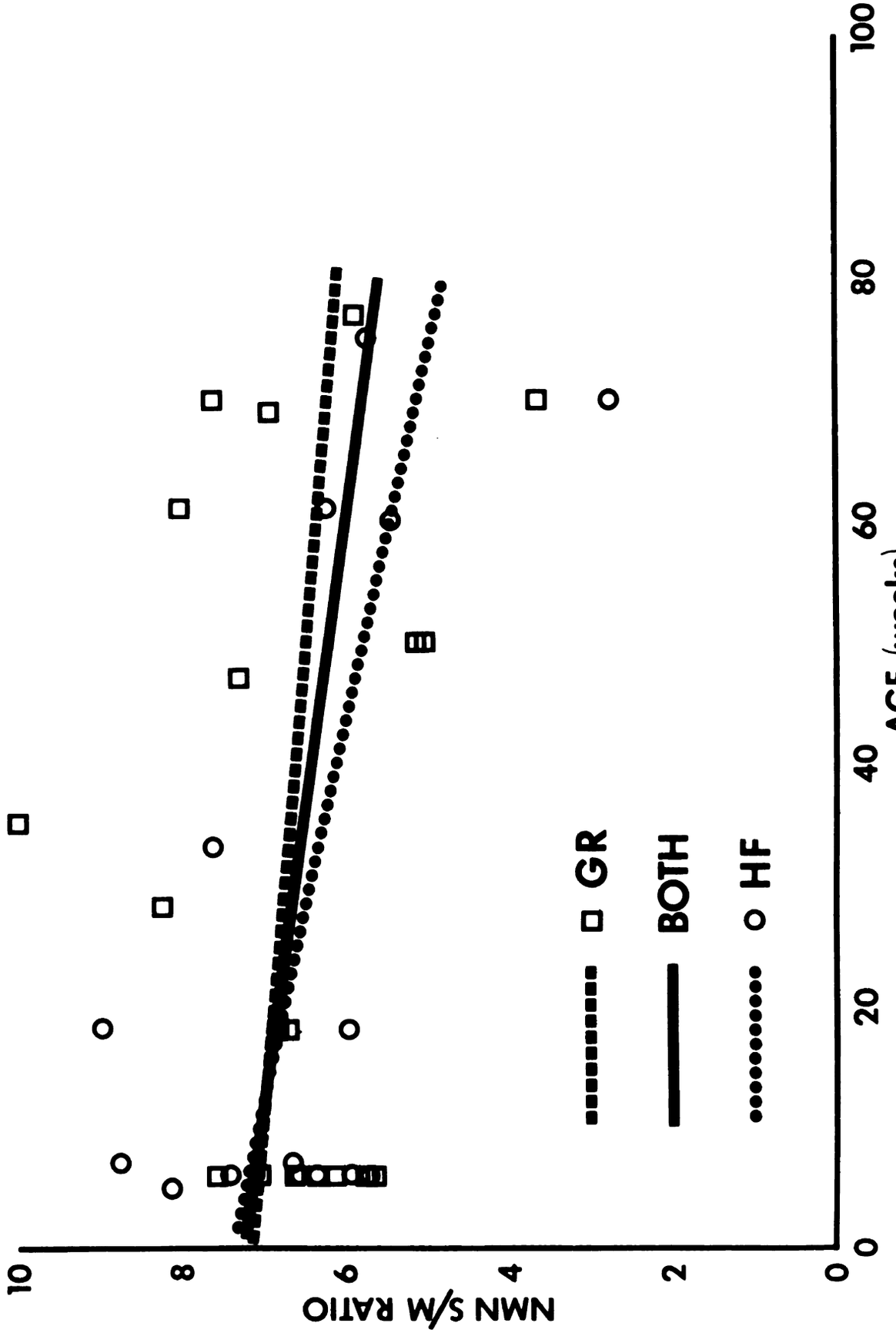


Figure 24

Figure 25. Accumulation of NMN (S/M ratio) in renal cortical slices from GR (□) and HF (○) rats plotted against kidney weight. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the average of duplicate determinations for individual rats. NMN accumulation in GR animals is not correlated with age ($P>0.05$). The other two lines demonstrate significant regression ($P<0.01$).

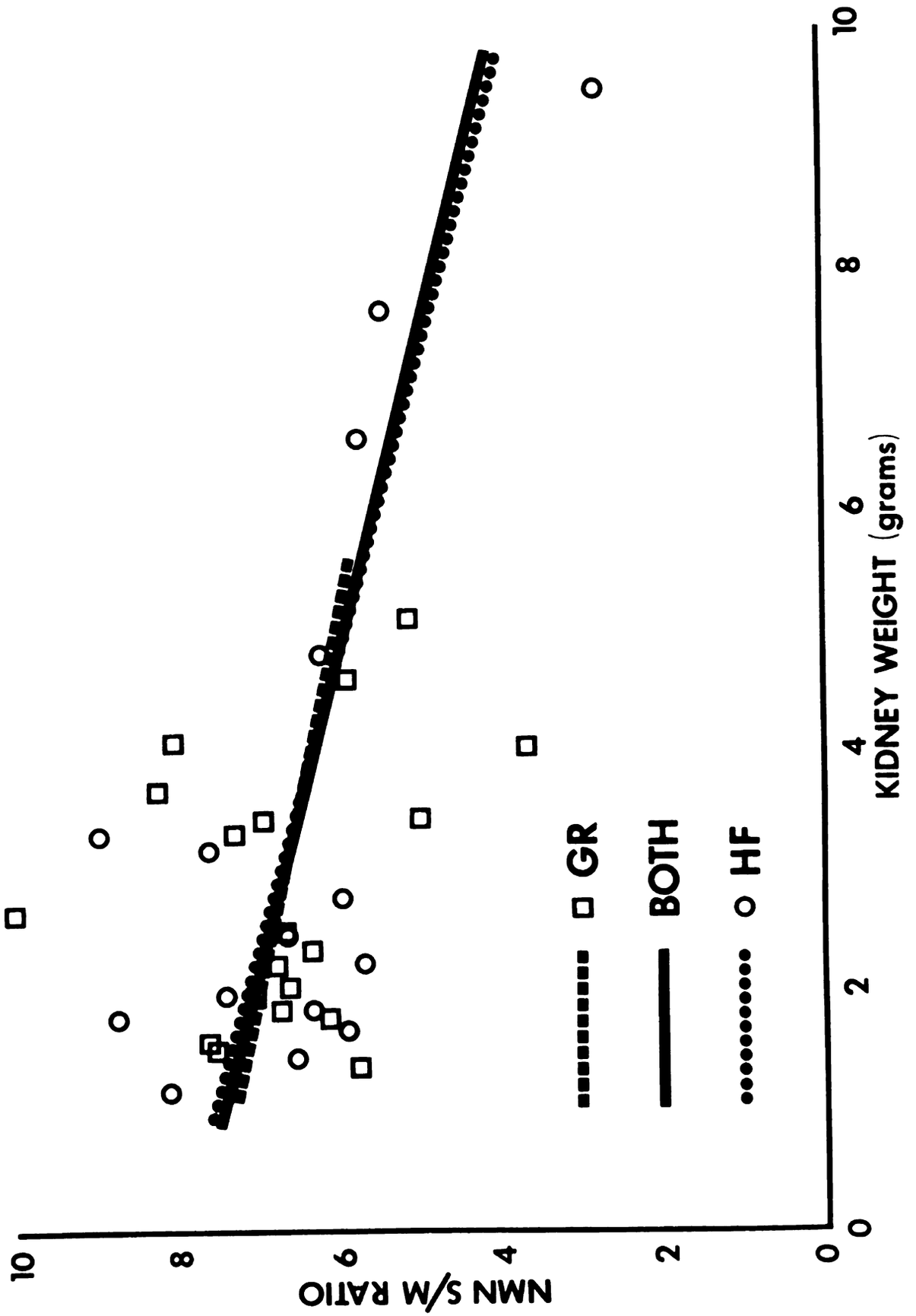


Figure 25

Figure 26. Accumulation of NMN (S/M ratio) in renal cortical slices from GR (□) and HF (○) rats plotted against body weight. The calculated regression lines for GR (■■■), HF (●●●) and all animals independent of diet (▬) are plotted. Points are the average of duplicate determinations for individual rats. None of the regressions are significant (P>0.05).

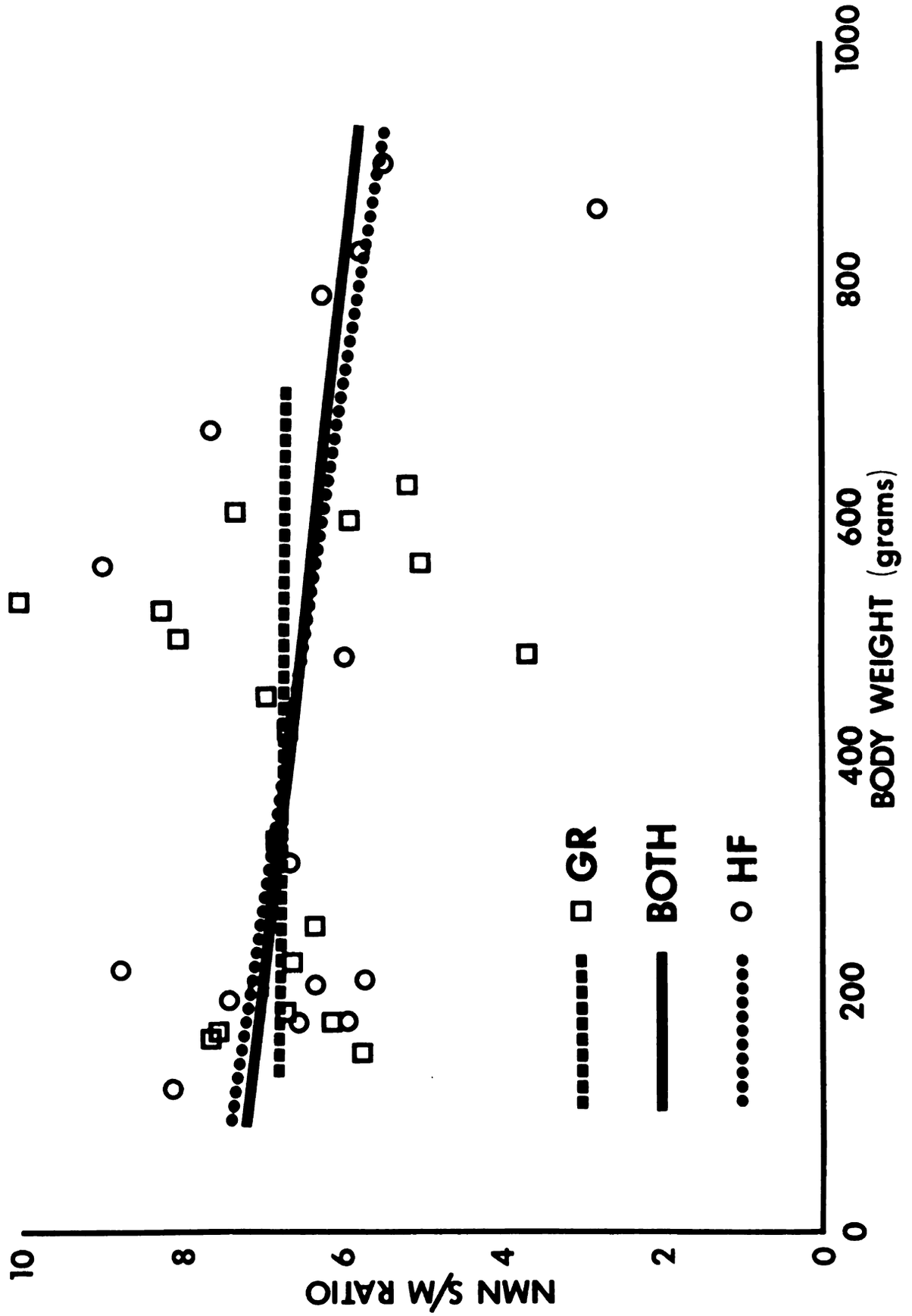

NMN S/M RATIO
10
8
6
4
2
0
0
200
400
600
800
1000
BODY WEIGHT (grams)
GR
BOTH
HF

Figure 26

Figure 27. Accumulation of PAH (S/M ratio) in renal cortical slices from male rats fed GR and switched to HF or fed HF and switched to GR. Each bar represents the mean ± (S.E.) obtained from duplicate determinations on the number of animals in parentheses. These values are significantly different from each other ($P<0.05$).

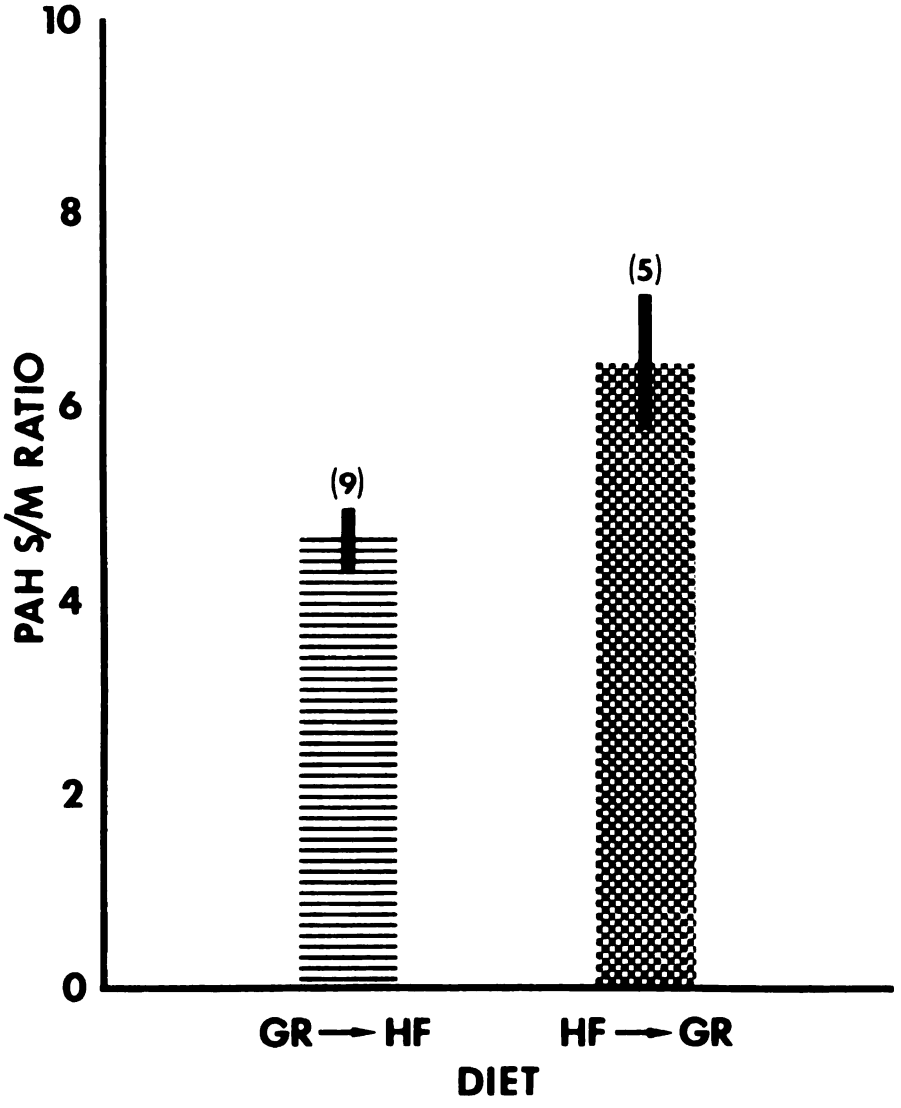


Figure 27

Figure 28. Accumulation of NMN (S/M ratio) in renal cortical slices from male rats fed GR and switched to HF or fed HF and switched to GR. Each bar represents the mean ± (S.E.) obtained from duplicate determinations on the number of animals in parentheses. These values are not significantly different from each other ($P>0.05$).

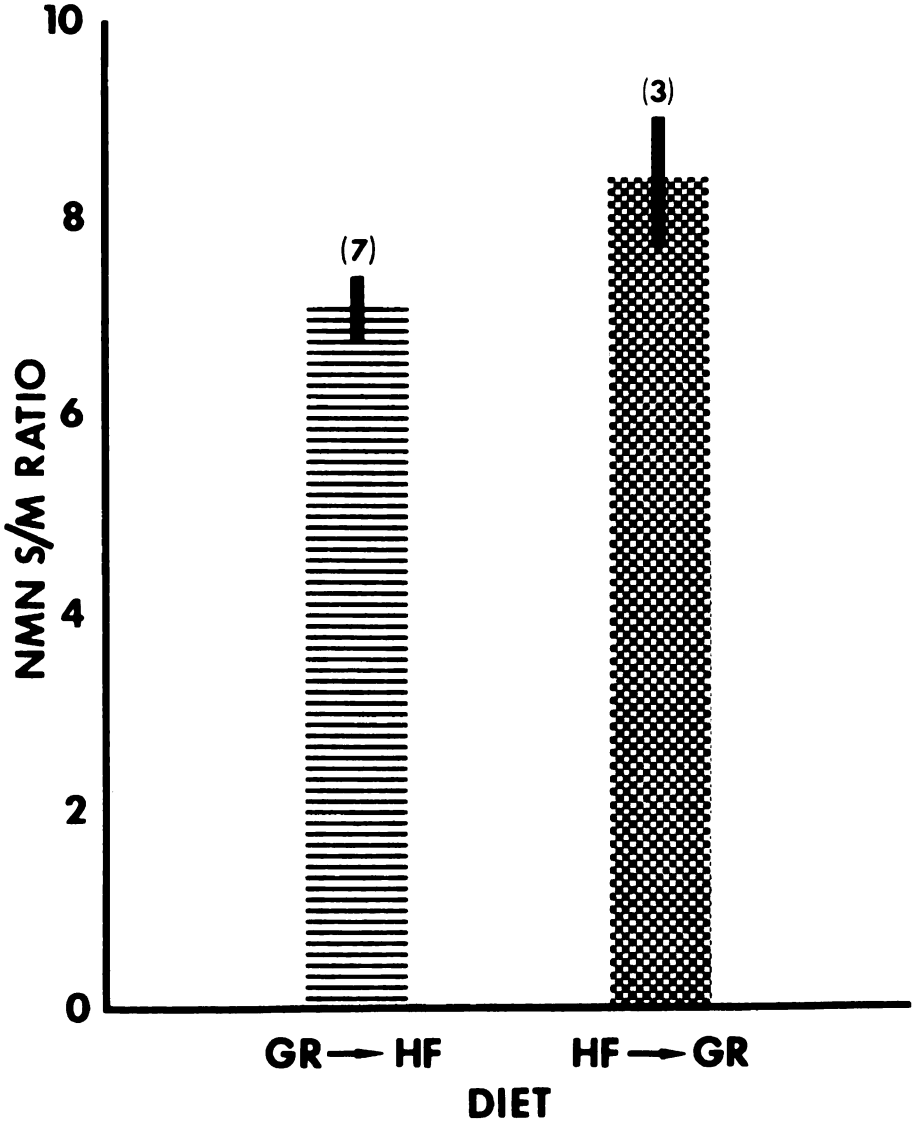
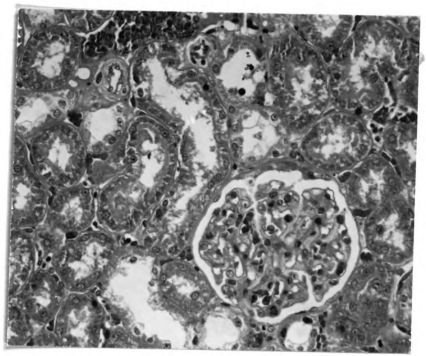


Figure 28

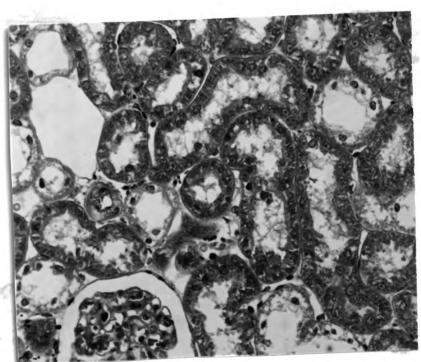
Figure 29. Histological sections of kidneys from HF-fed rats.

A&B: Kidneys from typical HF rats 30 weeks of age. Tubules are slightly dilated and lumens contain some proteinaceous debris. Glomeruli have normal basement membranes; Bowman's spaces are empty. Occasional glomeruli show metaplasia.

Measured PAH S/M Ratio: A - 3.75; B - 3.77. PAS stain, X256.



A



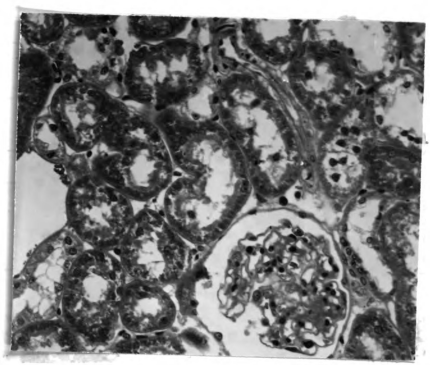
B

Figure 29

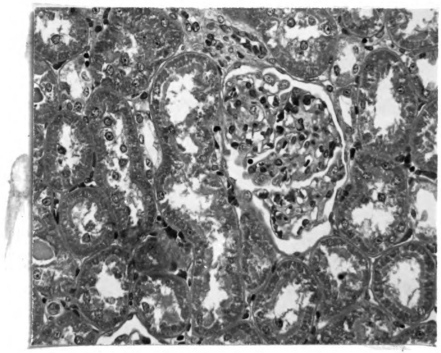
Figure 30. Histological sections of kidneys from GR-fed rats.

A&B: Kidneys from typical GR animals 30 weeks of age. Tubules are slightly dilated and contain some debris. Glomeruli have normal basement membranes and empty Bowman's spaces. Occasional glomeruli exhibit metaplasia, focal hypercellularity, and increased mesangial tissue.

Measured PAH S/M Ratio: A - 7.39; B - 8.51. PAS stain, X256.



A



B

Figure 30

Table 10. Effect of diet on the accumulation (S/M ratio)[a] of PAH by slices of rat renal cortex.

Diet[b]	Number[c]	PAH S/M Ratio[d]
GR	23	6.96±0.41
HF	18	4.81±0.29[e]
GR→HF	9	4.63±0.30[e]
HF→GR	5	6.48±0.73

[a]Ratios were determined after incubation at 90 minutes, at 25°C under 100% oxygen atmosphere.

[b]Diets used were Grain Ration (GR) and High Fat Ration (HF). GR and HF were fed throughout the study. GR→HF indicates the animal was fed GR and switched to HF prior to assay. HF→GR animals were fed HF and switched to GR prior to determining PAH accumulation. HF→GR and GR→HF animals were on the first diet from 1 to 45 weeks and on the second diet from 2 days to 3 weeks. PAH accumulation was unaffected by the length of time the first and second diets were fed; therefore, all values are pooled.

[c]Total number of animals on any regimen irrespective of age.

[d]For each animal duplicate determinations were made. These values were averaged and used to compute means and standard errors.

[e]Significantly different from GR ($P<0.001$).

Table 11. Effect of diet on the accumulation (S/M ratio)[a] of NMN by slices of rat renal cortex.

Diet[b]	Number[c]	NMN S/M Ratio[d]
GR	19	6.75±0.33
HF	15	6.55±0.40
GR→HF	7	7.03±0.34
HF→GR	3	8.36±0.65

[a]Ratios were determined after incubation for 90 minutes at 25°C under 100% oxygen atmosphere.

[b]The Grain Ration (GR) and High Fat Ration (HF) were fed throughout the study. GR→HF indicates the animal was fed GR and switched to HF prior to assay. HF→GR animals were fed HF and switched to GR prior to determining NMN accumulation. HF→GR and GR→HF animals were on the first diet from 1 to 45 weeks and on the second diet from 2 days to 3 weeks. NMN accumulation was unaffected by the length of time the first and second diets were fed; therefore, all values were pooled.

[c]Total number of animals on any regimen irrespective of age.

[d]From each animal duplicate determinations were made. These values were averaged and used to compute means and standard errors.

Table 12. Accumulation of PAH (S/M ratio)[a] by slices of rat renal cortex in the presence of serum.

Incubation Medium[b]	Serum Source	Kidney Source	Number[d]	PAH S/M Ratio
2.5 ml medium	HF[c]	GR[c]	5	15.38±0.21[e]
+ 0.5 ml serum	HF	HF	4	13.45±1.27[e]
	GR	GR	3	14.73±0.02[e]
	GR	HF	3	15.08±1.65[e]
2.5 ml medium	--	HF	2	4.25±0.02[e]
+ 0.5 ml saline	--	GR	2	7.28±0.25
2.7 ml medium	--	HF	18	4.81±0.29[e]
	--	GR	23	6.96±0.41

[a]Ratios were determined after incubation for 90 minutes at 25°C under 100% oxygen atmosphere.

[b]Cross and Taggart (1950) medium with PAH concentration of 7.4×10^{-5} M was used.

[c]Diets used were Grain Ration (GR) and High Fat Ration (HF).

[d]The number of experiments each representing one animal as a serum source and another as kidney source.

[e]Significantly different from control (GR kidneys in 2.5 ml medium + 0.5 ml saline) and from GR kidneys in 2.7 ml standard media ($P<0.05$).

Table 13. Kinetic analysis of PAH uptake in rat renal cortical slices[a].

Diet[b]	Age (wks.)	Slope[c]	K_m (10^{-4} moles/L)[c]	V_{max} (µg/g/min)[c]
GR	12	.391	15.62	39.92
HF	12	.360	5.37	14.92
GR	60	.490	6.10	12.45
HF	60	.318	1.47	4.63

[a]Rate of PAH uptake (µg PAH/g kidney slice/min) at PAH concentrations of 2, 4 and 8 x 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 minutes of incubation. Triplicate determinations of uptake for each diet and age were made.

[b]Diets used were Grain Ration (GR) and High Fat (HF) and were fed throughout the study.

[c]Values were obtained from the Lineweaver-Burk plot shown in Figures and .

Table 14. Effect of diet on pH of 24-hour urine samples from 10-week old male rats.

Diet[a]	Number	1 0-24 hrs	2 72-96 hrs	3 96-120 hrs
GR→GR	9	8.22±0.42	8.08±0.37	8.09±0.38
GR→HF	9	8.13±0.39	6.64±0.54[c]	6.39±0.45[c]

[a]Diets used were Grain Ration (GR) and High Fat Ration (HF).
GR→GR indicates GR was fed throughout the study.
GR→HF indicates GR was fed 0-24 hrs and HF afterwards.

[b]The time the initial urine collection was started was designated as 0 hr. Subsequent collections were timed accordingly.

[c]Values are significantly different ($P<0.05$) from those obtained from all animals in period 1 (0-24 hrs).

Table 15. Effect of diet on the pH of the medium after incubation[a] of renal cortical slices

Diet[b]	Number	pH
GR	4	7.41±.01
HF	4	7.36±.01[c]

[a]Incubations were for 90 minutes at 25° under 100% oxygen atmosphere. Immediately after removing the renal cortical slices, the pH of the medium was determined.

[b]Diets used were Grain Ration (GR) and High Fat Ration (HF) and were fed throughout the study.

[c]Significantly different from the control (GR) value ($P<0.05$).

Table 16. Oxygen consumption of renal cortical slices from rats fed the grain ration or high fat ration.

Diet	Number	µl/hr/mg tissue (wet)	µl/hr/mg (dry)
GR[a]	4	2.20±0.24	12.84±0.99
HF	4	2.52±0.09	11.97±1.32

[a]Diets used were Grain Ration (GR) and High Fat Ration (HF).

Table 17. Approximate composition of renal cortical slices from rats fed the grain ration or high fat ration.

Diet	Number	% Moisture	% Fat (Dry Wgt Basis)
GR[a]	9	86.56±0.69	8.28±0.60
HF	7	88.46±1.32	9.85±2.33

[a]Diets used were the Grain Ration (GR) and the High Fat Ration (HF).

DISCUSSION

Osborne-Mendel rats fed a high fat diet become grossly obese as determined by increased body weight or body fat (Schemmel *et al.*, 1969). They are useful as an experimental model for investigating the physiological effects of obesity which is a problem of major proportion in the United States. Armstrong *et al.* (1951), employing the tables of the Metropolitan Life Insurance Company, estimated about 15 million persons in the country were 10% overweight and at least 5 million are 20% or more above normal weight. Obesity has been called the most common nutritional disorder in the United States by Braunstein (1971), who estimates that 30% of the adult population is greater than 20% overweight. Recent reports linked obesity with an increased incidence or severity of various cardiovascular, skeletal, metabolic and organ abnormalities (Mayer, 1968; Armstrong *et al.*, 1951). The association of abnormal kidney function in obese humans was reported by several workers (Bittnerova *et al.*, 1968; Ross, 1960; Mayer, 1968).

An association of kidney damage with increased body weight was reported in hypothalamically obese rats (Brobeck *et al.*, 1943; Stevenson *et al.*, 1950 and Kennedy, 1957), in rats with unrestricted feed intakes (Berg and Simms, 1960; Bras and Ross, 1964) and in genetically obese rats (Zucker, 1965). Studies in our laboratory, reported

in this thesis, suggest that there are alterations in a number of kidney functions measured *in vivo* and as seen in renal histology.

Renal transport of organic acids is an important homeostatic mechanism as it is a means of excretion of potentially toxic by-products of metabolism (Pitts, 1968). Selleck and Cohen (1965) suggested that the primary function of the organic acid transport system is to move specific products of intermediary metabolism (nonesterified fatty acids, α-ketoglutarate, citrate, etc.) to sites of dissimilation in the kidney and liver. According to Goldberg *et al.* (1970), the organic acids (other than amino acids) in the urine are primarily derived from bacterial metabolism in the gastrointestinal tract. These include hippuric acids (Asatoor, 1965) and indolic compounds (Milne *et al.*, 1960). Endogenous production from phenylalanine accounts for a small portion of the hippuric acid in the urine (Armstrong *et al.*, 1955); however, the major portion is derived from dietary precursors (Armstrong *et al.*, 1955). The intestinal bacterial flora play an important role in the production of hippuric acid from dietary precursors (as benzoic acid and sodium benzoate) (Asatoor, 1965).

Organic acid transport is a specific function of the renal proximal tubule. This is in contrast to the renal handling of sodium which involves the entire length of the nephron. Sodium reabsorption is a general function

of the nephron and as such requires considerable expenditure of metabolic energy. Inasmuch as transport of organic acids is limited to the proximal tubule, it probably requires significantly less energy than sodium handling. Normally the sodium reabsorption mechanism is not operating at full capacity and is not challenged by the filtered sodium load. Thus a small decrement in energy availability might not affect overall sodium transport. In these experiments, it was possible to challenge the organic acid transport system by measuring uptake in the steady state. The rationale behind this was that when so challenged, subtle changes in this function might be observed prior to the appearance of physiological or biochemical lesions of the renal parenchyma. Transport of the organic acid, PAH, *in vitro* was determined in a Cross and Taggart incubation system. Thin renal cortical slices in a salt buffered, PAH-containing medium were incubated for 90 minutes under 100% oxygen at 25°C. Accumulation of PAH was expressed as the final slice concentration/medium concentration ratio (S/M ratio).

The accumulation of PAH (S/M ratio) was significantly depressed in HF animals when compared to GR animals (Figure 16). This was in agreement with PSP excretion *in vivo* in these animals. PAH transport was inversely correlated with age (Figure 17), body weight (Figure 18) and kidney weight (Figure 19) for both diets. Animals fed

HF were significantly heavier and had a higher percentage of body fat than GR animals of the same age (Schemmel *et al.*, 1969). Kidney weights were positively correlated with body weight in all animals (Figure 20). The depression of PAH accumulation by renal cortical slices with age confirms work of Barrows and co-workers (Adams and Barrows, 1963; Beauchene *et al.*, 1965). No reports regarding the effect of body weight on PAH transport were available in the literature. Numerous workers previously reported an association of a kidney weight with body weight (Hatai, 1913; Webster *et al.*, 1947; Widdowson and McCance, 1960).

Since PAH transport was depressed in the kidney slices from the HF animals, it became of interest to determine whether other tubular transport mechanisms were similarly altered.

Parallel systems for transport of organic acids and bases are located in the proximal tubules. The importance of the organic base transport system is less apparent, although a number of urine and plasma constituents are secreted by this system (Peters, 1960). Thiamine, choline, NMN (a nicotonic acid metabolite), guanidine, piperidine and methyl guanidine are naturally occurring compounds transported by the organic base system (Peters, 1960). This system might exist to secrete some unknown

substance(s), which because of high toxicity, must be maintained at very low plasma concentrations (Pitts, 1968).

The specificity of the depression of transport of organic acids in the HF group was determined using NMN as a prototype to study base transport. Workers have shown that the accumulation of PAH by renal cortical slices in the Cross and Taggart incubation system may be specifically depressed by a number of factors without any change in NMN transport (Hook and Munro, 1968; Hirsch and Hook, 1969b). Accumulation of NMN by renal cortical slices from animals fed GR and HF was not different. NMN transport was inversely related to age (Figure 24) and kidney weight (Figure 25) in the HF group. Body weight, however, was not significantly related to base transport (Figure 26). No explanation for the differences observed between the effect of age, body weight and kidney weight on transport of PAH and NMN in the animals is known. Other workers have suggested that the NMN transport system is more resistant to manipulation than the organic acid transport system (Hirsch, 1970; Bowman, 1970). These results suggested that the diet *per se* affects the accumulation of PAH by rat renal cortical slices.

When diets were switched, accumulation of PAH by renal cortical slices from animals fed HF for any period of time and switched to GR for any period of time just prior to sacrifice (Figure 27, Table 10) was similar to that by

kidneys from animals fed GR throughout (Table 10). Animals fed GR and switched to HF for any period of time had kidneys in which transport of PAH was significantly less than that for animals fed GR prior to sacrifice. Thus diet *per se* appeared to be a primary factor influencing the final S/M ratio, although age, body weight and kidney weight influenced the accumulation of PAH by renal cortical slices. These observations suggested that the effect of diet on the accumulation of PAH was easily reversible and the time required for this change was less than 2 days.

Since the effect of diet appeared readily reversible, it was reasoned that a dietary metabolite in the serum might be directly affecting the accumulation of organic acid in the *in vitro* system. However, when serum was incubated with the kidney slices, PAH accumulation was enhanced. Serum from HF animals stimulated the accumulation of PAH by kidney slices from both GR and HF animals (Table 12). Similarly, GR serum added to the incubation media significantly increased the accumulation of PAH by kidney slices from both groups (Table 12). These results confirm the effect of normal serum in enhancing PAH accumulation by renal cortical slices reported by Orringer *et al.* (1971).

Thus, if some factor of dietary origin in the serum of the HF animals is depressing PAH accumulation by renal

cortical slices, its concentration is insufficient to produce an inhibitory effect when added to the incubation system. Apparently, the factor(s) responsible for the enhancement of PAH accumulation is in adequate concentration to overcome any inhibitor that might be present in the HF serum. This is in contrast to reports that "uremic" serum (White, 1966; Preuss *et al.*, 1966) or serum from nephrectomized animals (Hook and Munro, 1968), when added to this incubation system, depressed the accumulation of PAH by renal cortical slices.

The depressed PAH accumulation of kidney slices from HF rats could be increased to a level equal to that of slices from GR rats by the addition of the serum. This suggests that there is no difference in the inherent functional capacities of the kidneys from the obese and control rats for PAH transport. Since differences in other kidney functions are reported in HF animals, this is a significant result.

The factor(s) in the serum enhancing the transport has not been elucidated. Conceivably, the effect could be mediated by an increased or preferential energy source (glucose, acetate, etc.) and/or a more favorable electrolyte balance. In addition, the protein in the serum could possibly aid in maintaining the cellular integrity of the slice during the incubation period.

The depressed PAH accumulation in kidneys from HF animals could result from a number of contributing factors.

These include differences in entry, accumulation and/or runout of PAH, in non-specific binding of PAH to protein, and in extracellular water, intracellular water or protein content of the renal cortical slice.

The renal transport system as studied is not a pure system and renal organic acid transport probably does not follow true Michales-Menten (M-M) kinetics. Nevertheless, the Lineweaver-Burk plot remains one of the most useful tools available to study these transport kinetics (Farah *et al.*, 1959; Nagwekar and Unnikrishnan, 1971; Orringer *et al.*, 1971). An analysis of the inhibition of the transport system using a Lineweaver-Burk plot is useful in that its findings are consistent with results obtained from the intact animal. Also, it aids in predicting how a new inhibitor may function.

The S/M ratio reported in this study was measured in a steady state system and thus represents binding and runout capacity of the renal tissue as well as transport capacity. Therefore, the S/M ratio is a measure of the ability of the tissue to maintain a concentration gradient. During short periods of incubation (as between 2-12 minutes) uptake of PAH is linear, suggesting that intracellular accumulation of the compound is not sufficiently high to alter the rate of influx. The uptake over short periods of time indicates the rate of transport of a material into the tissue, according to Ross *et al.*

(1968b). In the Lineweaver-Burk plot, the x-intercept ($1/K_m$) could reflect the relative affinity of a carrier substance for PAH and the y-intercept ($1/V_{max}$), the maximal velocity of the PAH accumulation process. On the basis of these assumptions the data presented in Figure 17 and Table 13 suggest that the apparent affinity and maximal velocity are both different when values for older GR animals are compared with older HF animals. These changes in kinetics suggest non-competitive inhibition. Competitive inhibition was suggested when PAH S/M ratios were depressed after adding fasting serum to the incubation media (Hook and Munro, 1968). Another interpretation and probably the more likely is that the inhibitor-carrier interaction is nonreversible or slowly reversible and that M-M kinetics are not applicable. This inhibitor may be in such low concentrations that no effect is seen in acute studies. Apparently the inhibitor was not released even after the renal cortical slices remained in saline or the Cross and Taggart medium for two hours. In the intact animal, an inhibitor could accumulate and eventually impede the transport system. Such a possibility was suggested by Balagura-Baruch and Stone (1969) in dogs. These workers reported that α-ketoglutarate inhibited PAH secretion by a noncompetitive or mixed mechanism.

The possibility that the acidity of the urine could influence PAH accumulation was also considered. This was

based on the fact that when the kidneys were removed from the animals, they contained small amounts of urine, the composition of which was similar to that examined for acidity. Several workers have reported the susceptibility of the acid transport system to the pH of the incubation medium (Bowman, 1970; Forster and Copenhaver, 1956). A group of 10-week old animals fed GR for 7 weeks had 24 hour urinary samples with a pH of 8.18±0.37 (Table 18). Nine of the original group after switching to HF for one day had urine samples for the 24 hour collection with a pH of 6.64±0.54 (Table 18). Recognizing that diets of different composition may produce acid, neutral or alkaline ash, and that urinary pH is not as valid a measure of H^+ secretion as are others (i.e., titratable acidity), the contribution of urinary pH to the depression of PAH accumulation in kidneys from HF animals was doubtful. Also, despite these differences in urine pH of the animals fed the different rations, it is doubtful whether these are responsible for the observed changes in the S/M when the rations were switched. Furthermore, if tubular urinary pH is important, perhaps the pH of the incubation media is different after incubating HF slices compared to GR. The pH of the medium immediately after 90 minutes incubation was measured and found to be significantly different (Table 19). However, the physiological significance of this difference in pH is

questionable. Copenhaver and Davis (1965) reported the accumulation of PAH by rat renal slices was not different between pH 7.3 and 7.4.

Differences in accumulation of PAH by renal cortical slices from HF and GR rats was not reflected in changes in oxygen consumption (Table 20). This is in agreement with a number of reports (Taggart *et al.*, 1953; Burg and Orloff, 1962a; Copenhaver and Davis, 1965; Bourke *et al.*, 1967; Chung *et al.*, 1970). These results are in contrast to those reported by Huang and McIntosh (1955).

The fat and moisture percentage compositional changes were not significantly different in the two groups (Table 21).

No apparent morphological differences were observed in renal cortical sections from HF and GR animals by light microscopy (Figure 29, Figure 30). Although renal cortical slices from GR animals obtained PAH S/M ratios which were significantly greater than those developed by slices from HF animals, the slices could not be distinguished histologically (Figure 29, Figure 30). Similarly, Hirsch *et al.* (1971) observed no histological differences between kidneys from penicillin-treated and control rabbits although penicillin treatment resulted in a significant increase in PAH accumulation. There may, however, be precise differences in ultrastructure or enzyme activity which could be determined by electron microscopy or histochemically.

SPECULATION

Intuitively one would consider that the high fat content of the diet of the obese animals was the factor responsible for the depression of PAH accumulation by rat renal cortical slices. Before such an assumption is accepted, it should be recognized that the HF and GR rations differ in other respects. The HF ration is composed primarily of purified ingredients and a hydrogenated vegetable fat, whereas the GR ration contains primarily corn and soybean meal.

Discrete subtle differences in the diets and their effects on accumulation of PAH as determined in these experiments must be considered before the dietary component(s) or metabolite(s) contributing to depression of PAH transport can be determined. This could be of special significance if in fact the component(s) is irreversibly binding the sites involved in PAH transport. One of the problems in attempts to isolate the PAH carrier system has been a lack of a specific inhibitor which irreversibly blocks PAH transport (as dibenamine blocks NMN transport). Characterization of the blocker in the HF diet could conceivably provide a compound useful in the isolation of the carrier system.

Nonesterified fatty acids (NEFA) from the HF diet could also be affecting accumulation of PAH in renal cortical slices in this incubation system. Inhibitors of

organic acid transport, probenicid (Huang and Lin, 1965) and chlorothiazide (Beyer and Baer, 1961), also inhibit net renal NEFA utilization (Barac-Nieto and Cohen, 1968). Cohen (1964) reported that α-ketoglutarate, a inhibitor of organic acid transport (Cross and Taggart, 1950) inhibits net renal NEFA uptake. NEFA are bound to proteins (Goodman, 1958a), to cell membranes, (Goodman, 1958b), and to intracellular particles (Reshef, 1966). Conceivably, NEFA could be an inhibitor in this study. This should be further investigated.

The factor(s) in the serum which enhance the accumulation of PAH by renal cortical slices are unknown. The serum contains energy sources, acetate ions, and electrolytes known to be involved in the transport process or to stimulate it. Also, whether the addition of serum or metabolites (as ATP) would overcome the depressed PAH accumulation in slices from older animals or anoxic slices is not known.

SUMMARY

The effect of obesity and a high fat diet (HF) on the accumulation of PAH by renal cortical slices was determined using the *in vitro* slice technique of Cross and Taggart (1950). The accumulation of PAH was significantly depressed in animals fed the HF diet used to produce obesity. Accumulation of PAH decreased with increasing age, body weight and kidney weight independent of diet. Similarly, kidney weight was significantly correlated with body weight in HF and GR animals.

The rate of PAH uptake was determined and analyzed kinetically using a Lineweaver-Burk plot. GR animals at 12 weeks of age exhibited a higher V_{max} and K_m indicative of greater velocity and apparent affinity. In older GR animals (60 weeks), the V_{max} and K_m were greater than that of the younger and older HF animals, but less than that of the young GR animals.

Organic base accumulation was determined in order to demonstrate the specificity of the effect of the HF diet and obesity on organic acid transport. Accumulation of NMN was not different in GR and HF animals. There was no correlation of NMN accumulation with body weight. In HF animals NMN accumulation by renal cortical slices decreased with increased age and kidney weight. Age and kidney weight in the GR animals were not related to NMN accumulation.

Exchanging the diets for a few days or a few weeks affected the transport of PAH, but not NMN transport. Animals fed GR for any period of time immediately prior to sacrifice had kidneys which accumulated PAH significantly more than those fed HF just prior to sacrifice.

Addition of HF or GR serum to the incubation medium enhanced PAH accumulation by kidney cortical slices from GR or HF animals. The differences observed in PAH transport in the HF animals did not result in differences in oxygen consumption by renal cortical slices. Histologically HF kidney slices were not different from GR kidney slices although PAH S/M ratios were significantly different. Although composition of slices from GR and HF animals were slightly different, the extent that these differences would affect the PAH transport system is not known.

These data suggest that PAH transport is depressed in animals with increased age, body weight and kidney weight. Furthermore, PAH transport may be depressed by dietary manipulation, feeding a 60% fat diet. These data emphasize the importance of carefully considering the diet, age, and body weight in studies of renal function in rats.

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APPENDICES

APPENDIX A

Grain Ration (Campbell *et al.*, 1966)

Component	Amount/100 gm.
ground corn	60.70 gm.
soybean meal	28.00 gm.
alfalfa meal	2.00 gm.
fish meal	2.50 gm.
dried whey	2.50 gm.
limestone	1.60 gm.
dicalcium phosphate[1]	1.75 gm.
iodized salt	0.50 gm.
mineral mix[2]	.116 gm.
vitamin mix[3]	.127 gm.
penicillin	0.20 mg.
streptomycin	0.80 mg.
arsenilic acid	96.80 mg.
vitamin A	801 I.U.
vitamin D_2	75 I.U.

[1]Feed grade $CaHPO_4 \cdot 2H_2O$, proximate analysis 18.5% P, 22-25% Ca.

[2]Percentage composition of mineral mix: $MnSO_4 \cdot H_2O$, 32.06; $FeSO_4 \cdot 7H_2O$, 40.83; $CaCO_3$, 15.72; $ZnCO_3$, 7.62; $CuSO_4 \cdot 5H_2O$, 2.45; $CoCl_2 \cdot 6H_2O$, 0.90; KI, 0.43.

[3]Percentage composition of vitamin mix: choline chloride, 55.13; calcium pantothenate, 0.43; riboflavin, 0.26; niacin, 2.60; vitamin B_{12} (0.1% mannitol triturition), 0.52; α-tocopherol acetate (250 I.U.P. per gm.), 1.53; menadione, 0.17; DL methionine, 39.36.

APPENDIX B

High Fat Ration (Mickelsen *et al.*, 1955; Schemmel *et al.*, 1969a)

Component	Amount/100 gm.
shortening (*Crisco*)	60.00 gm.
casein	25.00 gm.
non-nutritive fiber	2.00 gm.
aureomycin	0.01 gm.
liver mix	2.00 gm.
DL methionine	0.25 gm.
sucrose	3.54 gm.
mineral mix[1]	5.00 gm.
vitamin mix[2]	2.20 gm.

[1]Percentage composition of mineral mix: $CaCO_3$, 21.0; $Ca_3(PO_4)$, 14.9; $CuSO_4 \cdot 5H_2O$, 0.039; $FePO_4 \cdot 4H_2O$, 1.47; $MgSO_4$, 9.00; $MnSO_4$, 0.02; $K_2Al_2(SO_4)_4 \cdot 24H_2O$, 0.009; KCl, 12.0; KI, 0.005; KH_2PO_4, 31.0; NaCl, 10.5; NaF, 0.057.

[2]Percentage composition of vitamin mix: vitamin A concentrate (200,000 U.S.P./gm.), 0.45; vitamin D concentrate (400,000 U.S.P./gm.), 0.025; α-topocerhol, 0.50; ascorbic acid, 4.50; i-inositol, 0.50; choline chloride, 7.50; menadione, 0.225; para-amino benzoic acid, 0.50; niacin, 0.45; riboflavin, 0.10; pyridoxine HCl, 0.10; calcium pantothenate, 0.30; biotin, 0.002; folic acid, 0.009; vitamin B, 0.0135; sucrose, 84.75.

APPENDIX C

Modified Grain Ration (Smith, 1969)

Component	Amount/100 gm.
ground corn	61.6 gm.
soybean meal	28.4 gm.
alfalfa meal	2.0 gm.
fish meal	2.6 gm.
dried whey	2.6 gm.
limestone	0.17 gm.
dicalcium phosphate[1]	1.75 gm.
iodized salt	0.5 gm.
mineral mix[2]	116 mg.
vitamin mix[3]	127 mg.
penicillin	0.2 mg.
streptomycin	0.8 mg.
arsenilic acid	96.8 mg.
vitamin A	801 I.U.
vitamin D_2	75 I.U.

[1]Feed grade $CaHPO_4 \cdot 2H_2O$, proximate analysis 18.5% P, 22-25% Ca.

[2]Percentage composition of mineral mix: $MnSO_4 \cdot H_2O$, 32.06; $FeSO_4 \cdot 7H_2O$, 40.83; $CaCO_3$, 15.72; $ZnCO_3$, 7.62; $CuSO_4 \cdot 5H_2O$, 2.45; $CoCl_2 \cdot 6H_2O$, 0.90; KI, 0.43.

[3]Percentage composition of vitamin mix: choline chloride, 55.13; calcium pantothenate, 0.43; riboflavin, 0.26; niacin, 2.60; vitamin B_{12} (0.1% mannitol trituration), 0.52; α-tocopherol acetate (250 I.U.P. per gm), 1.53; menadione, 0.17; DL methionine, 39.36.

APPENDIX D

Modified High Fat Ration (Smith, 1969)

Component	Amount/100 gm.
shortening	56.6 gm.
casein	23.6 gm.
non-nutritive fiber	2.0 gm.
aureomycin	0.01 gm.
liver mix	1.89 gm.
DL methionine	0.240 gm.
sucrose	3.34 gm.
dicalcium phosphate[1]	2.97 gm.
mineral mix[2]	5.0 gm.
vitamin mix[3]	2.2 gm.
metaphosphoric acid	0.27 gm.
magnesium carbonate	1.96 gm.

[1]Feed grade $CaHPO_4 \cdot 2H_2O$, proximate analysis 18.5% P, 22-25% Ca.

[2]Percentage composition of mineral mix: $CaCO_3$, 21.0; $Ca_3(PO_4)$, 14.9; $CuSO_4 \cdot 5H_2O$, 0.039; $FePO_4 \cdot 4H_2O$, 1.47; $MgSO_4$, 9.00; $MnSO_4$, 0.02; $K_2Al_2(SO_4)_4 \cdot 24H_2O$, 0.009; KCl, 12.0; KI, 0.005; KH_2PO_4, 31.0; NaCl, 10.5; NaF, 0.057.

[3]Percentage composition of vitamin mix: vitamin A concentrate (200,000 U.S.P./gm.), 0.45; vitamin D concentrate (400,000 U.S.P./gm.), 0.025; α-tocoperhol, 0.50; ascorbic acid, 4.50; i-inositol, 0.50; choline chloride, 7.50; menadione, 0.225; para-amino benzoic acid, 0.50; niacin, 0.45; riboflavin, 0.10; pyridoxine HCl, 0.10; calcium pantothenate, 0.30; biotin, 0.002; folic acid, 0.009; vitamin B, 0.0135; sucrose, 84.75.

APPENDIX E

Parameters Evaluated in Histological Sections of Kidneys

Capsule
- Thickened
- Irregular

Glomerulus
- Bowman's Capsule
  - Thickened
  - Fibrosis
  - Hyaline
  - Crescent
  - Necrosis
  - Irregular
- Glomerular Tuft
  - Broken
  - Thickened
  - Atrophic
  - Adhesions
  - Fibrosis
  - Degeneration
- Bowman's Space
  - Fibrosis
  - Blood
  - Protein
  - Enlarged

Proximal Tubule
- Epithelium
  - Hyperplasia
  - Degeneration
  - Necrosis
  - Cystic with Filtrate
  - Swelling
  - Cellular Casts
  - Fibrosis
- Basement Membrane Thickened

Neutrophils

Lymphocytes

Henle's Loop
- Cells and Protein Casts
- Hyaline Casts
- Hemoglobin Casts

Corticomedullary Junction
- Cystic Tubules with Filtrate
- Protein Debris in Tubules
- Tubular Necrosis

Collecting Tubules
- Casts
- Epithelium Degeneration
- Hemoglobin and Protein in Lumen

Renal Papillae
- Protein in Tubules
- Necrosis
- Hemorrhage

Renal Pelvis
- Hemorrhage

Miscellaneous
- Calcification
- Nephritis
- Hemorrhage, Focal
- Fibrosis
- Infarction