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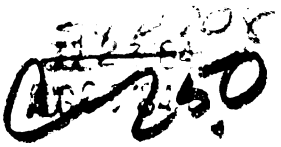

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THE EFFECTS OF OVERFEEDING DURING SUCKLING ON THE DNA, PROTEIN AND TRIGLYCERIDE CONTENT OF THE KIDNEYS IN TWO STRAINS OF RATS

By

Marianne Stone

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ABSTRACT

THE EFFECTS OF OVERFEEDING DURING SUCKLING ON THE DNA, PROTEIN AND TRIGLYCERIDE CONTENT OF THE KIDNEYS IN TWO STRAINS OF RATS

By

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Male Osborne-Mendel and S 5B/P1 rats were overfed from birth to 24 or 105 days. The effects of feeding a 44% or a 3% (w/w) fat diet, supplementary feeding during the suckling period and reduced litter size were observed. The overfeeding technique which exerted the greatest effect on growth was feeding a high fat diet. In comparison to rats fed the low fat diet, kidney DNA and protein were significantly elevated in Osborne-Mendel rats of both ages fed the high fat diet. The kidneys of these rats contained more cells than the controls, although cell size was not different. The effect on growth of overfeeding S 5B/P1 rats was negligible, although kidney DNA was elevated at 24 days and triglyceride at both ages in the S 5B/P1 experimental group. The increase in kidney weight with age in both strains was due to a doubling in the number of cells and in the protein/DNA ratio.

To Gary,
Gary Lee and
David

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REVIEW OF LITERATURE

Growth of the Kidneys, in General

Embryologically, the mammalian kidney passes through three stages of development; the pronephros, the mesanephros and the metanephros. These kidneys develop successively, one to another, with the gradual disappearance of the pronephros and mesanephros. The permanent kidney of the adult mammal is the metanephros.

The tubules of all three kidneys arise from the mesoderm of the intermediate cell mass or its extension, the nephrogenic cord (Allen, 1951). The vesicles of the pronephros begin to develop in human embryos at about the twentieth day, and disappear nearly as soon as they are formed. The mesanephros, or Wolffian Body, develops caudally to the pronephros beginning about twenty-four days prenatally. It attains its maximum number of sixty glomerular-tubular units by five to six weeks (Osathanondh and Potter, 1963a or Potter and Osathanondh, 1966, p. 1). About this time, a ureteral bud arises from each mesenphric duct. The buds grow and penetrate a mass of cells called the metanephric blastema. Interaction between these two entities causes the development of the metanephric kidney (Potter and Osathanondh, 1966). By the fourth prenatal month the mesanephros has degenerated.

The vesicles of all three kidneys contain glomeruli, tubules and a main duct. The nephrons of the final kidney, however, are much more highly organized and complex than the first two kidneys. In man, there

is a time during the early development of the metanephros when the nephrons of both the mesanephros and metanephros are active. The functioning of the mesanephric kidney in various species appears to be related to the efficiency of the placenta (Edelmann and Spitzer, 1969). The pig has an inefficient placenta, but a very well functioning mesanephros.

The rat has an efficient hemochorial placenta and a corresponding non-functioning mesanephros. The situation in humans is similar to that of the rat since, although the mesanephros is functional, it degenerates quickly (Edelmann and Spitzer, 1969).

The Metanephric Kidney

The metanephric kidney develops from two types of cells; those of the ureteric buds, and those of the metanephric blastema. The two ureteric buds, which arise from the mesenphric ducts, give rise to the ureter, renal pelvis, major and minor calyces, and collecting tubules. The anterior portion of each bud, the ampulla, grows actively in length and by dichotomous branching (Osathanondh and Potter, 1963a, 1963b), and is the end which induces nephrogenesis. Ampullary growth and the formation of new nephrons is complete by 32-36 weeks in the human fetus. The characteristics of ampullary growth are anterior extension, division, induction of nephrons and establishment of communication with nephrons (Potter, 1972).

The lower portion of the ureteric bud is the interstitial portion, the cells of which do not highly differentiate. Growth in this section continues until the full size of the kidney is attained, and is reflected as an increase in the length of the tubules (Potter, 1972)

The cells of the metanephric blastema differentiate into either nephrogenic or stromogenic cells which give rise to nephrons and connective tissue, respectively. Differentiation is brought about when the cells are penetrated by the ampulla. Those blastema cells near the growing, dividing ampulla become the origin of the nephrons, while those near the interstitial portion of the bud become connective tissue.

The dichotomous branching of the ampullae gives the kidney its basic structural arrangement. The first few divisions are species specific, and establish the number of lobes that each kidney will contain. The kidneys of such mammals as rat, rabbit and cat have only one lobe, in which a single papilla protrudes into a space which joins the ureter (Potter, 1972). The kidneys of man and other primates are multilobed. Potter (1972) has established that the first three to five generations of tubules produced by ampullary division dilate to form the renal pelvis, the next three to five generations dilate to form the minor calcyces, papillae and cribiform plates, and the next six to nine generations form the collecting tubules.

In the human fetus, the capacity of the ampullae for nephron induction is apparent from the eighth week until the thirty-second or thirty-sixth week. Nephrogenesis first begins when the ureteral bud has from twenty to forty terminal branches (Potter and Osathanondth, 1966). The blastema cells near the end of the ampulla condense, or aggregate, and become less mobile. Using time lapse photography, Saxen et al. (1965) demonstrated the existence of a trapping effect of the aggregated cells upon the more mobile blastema cells. The aggregate cells grow in this way by trapping more cells, and by cell proliferation. Then the aggregate assumes a spherical formation, with radially oriented wedge-shaped

cells (Saxen et al., 1968). A small cavity appears and the cells elongate, forming a single layer. The structure then takes on a characteristic S-shape. Upon further differentiation, one end of the vesicle communicates with the ampulla, and the other end forms a spoon-shaped double layer of cells. The cells of the outer layer become thin and flat, and form Bowman's Capsule. The other layer of cells becomes tall and narrow (columnar), and forms the epithelial layer of the glomerulus. When the capillary loop is evolved and grows close to these columnar cells, it also moves a group of connective tissue cells into the glomerulus. These connective tissue cells are thought to persist as mesangial cells (Potter and Osathanondh, 1966, p. 3).

During its existence, the activity of the ampulla is variable. Potter (1972, pp. 29-43) and Potter and Osathanondh (1966; Osathanondh and Potter, 1963b) have defined four periods in the development of the human kidney, based on changes in ampullary activity:

> Period 1 extends from the fifth to the fourteenth or fifteenth week prenatally. It begins when the ureteric bud arises from the mesenphric duct, and includes the eighth week when nephrogenesis begins. At the beginning of nephrogenesis, the ampulla induces the formation of two nephrons. The ampulla divides and each branch carries with it, one nephron. Upon dividing thereafter the ampulla is capable of inducing only one nephron. During Period 1, an ampulla which already has an attached nephron cannot induce the formation of another. Only a freshly divided ampulla can induce nephrogenesis. This period may be identified microscopically by the observation that the nephron attaches to the ampulla before the ampulla grows very far from

the point of branching.

Period 2 extends from week fourteen or fifteen until week twenty or twenty-two of fetal growth. It is known as the period of arcade formation. An arcade is a succession of from two to eight (usually four) nephrons which drain into a single collecting tubule. During this time there is little ampullary branching, and the function of nephron induction is carried on by ampullae which already have an attached nephron. Unlike Period 1, ampullae with attached nephrons can induce nephrogenesis. The result is a succession, or arcade, of nephrons only the youngest of which is attached to the ampulla. The connecting tubules of each nephron in the arcade merge into that of the youngest nephron. The forward growth of the ampulla, at this time, is due to growth in the interstitial portion of the ureteral bud.

Period 3 extends from twenty or twenty-two weeks until thirty-two to thirty-six weeks prenatally. It is responsible for the formation of the unbranched terminal portions of the collecting tubules, and for the nephrons whose glomeruli are located in the outer half of the cortex. The ampullae again grow by local cell proliferation, although they seldom branch. They grow beyond the arcade formations, and beyond any nephrons formed during this period. The ampulla induces nephrogenesis only when not carrying an attached nephron, and the nephrons which are formed become attached to the interstitial portion of the tubule.

Period 4 extends from the thirty-second to thirty-sixth week prenatally until adult life. The ampullae disappear at this time, and there is no further terminal growth or branching of the collecting tubules. The induction of nephrons ceases. Any changes which occur are due to interstitial growth and cellular differentiation.

By the time of birth, then, the kidneys of the neonate contain an extensive tubular system, and essentially all of their nephrons. From thirty-two to thirty-six weeks prenatally until adulthood the ureters and collecting tubules grow in length, the cells of the metanephric blastema continue to differentiate into connective tissue, the vascular system elaborates, and the nephrons grow and differentiate. The nephrons grow primarily by an increase in the length of their tubules, especially in the areas of the proximal tubule and the Loop of Henle. The glomeruli of the adult have only a slightly greater diameter than those of the infant or fetus (Allen, 1951). As growth continues the nephric tubule becomes more coiled, or tortuous, especially in the proximal portion.

The cortex of the mature kidney is composed of terminal branches of collecting tubules, glomeruli, proximal and distal tubules and the portions of the Loops of Henle which lie near the collecting tubules. The medula contains collecting tubules and the remaining portions of the Loops of Henle (Potter and Osathanondh, 1966).

Indices of Growth

There was considerable interest early in history regarding the growth and development of the kidneys. It had been noticed that in

persons born with one kidney, the organ was considerably enlarged. In the same context, Nowinski and Goss (1969) have given an interesting account of the historical development of the unilateral nephrectomy and research that has evolved concerning the growth of the remaining kidney. In 1871, Rosenstein (as cited in Nowinski and Goss, 1969) noted that a difference normally exists in the weight of the left and right kidney. This has been a controversial topic for several years with some investigators reporting that the right kidney in rats is larger than the left (Arataki, 1926; Coe and Korty, 1967; Halliburton, 1969), and others finding no difference (Smith and Moise, 1927; Zumoff and Pachter, 1964; Mason and Ewald, 1965).

Not trusting weight to be the only criterion of growth, Ribbert, in 1882 (as cited in Nowinski and Goss, 1969), measured kidney volume by its displacement of water in a measuring cylinder and also measured the diameters of glomeruli microscopically. In 1917, Kittelson studied the relative growth of the cortex and medulla at different ages in rat kidney by tracing serial sections of kidney on paper and then cutting and weighing them. The weight of the paper was converted to surface area, and then to volume. Kittelson also used tracings to count the number of glomeruli and to calculate their volume. In the late 1920's and early 1930's, MacKay et al. (1927a, 1927b, 1931) used weight and surface area as criteria of growth when experimenting with the effects of age and diet on the kidney. Donaldson (1924) and Dunn et al. (1947) measured individual organ and total body weight and length in studies of normal growth in the rat.

Although weight gain is a valuable parameter in the assessment of growth, it is disadvantageous in that weight can increase in the

absence of growth, such as with the accumulation of fat and water. In 1960, Widdowson and Dickerson studied the growth of organs and tissues by using another approach. Chemical maturity was assessed at various ages in pig and man by analyzing organs and tissues for water, total nitrogen, sodium, potassium, chloride, phosphorus, magnesium and calcium. Later, Widdowson and coworkers (1971, 1972) expanded their chemical composition studies to include the analysis of deoxyribonucleic acid (DNA).

It is now possible to use weight gain data in conjunction with more specific growth parameters at the cellular level. An organ or tissue grows by increasing its number of cells, by enlarging its existing cells and by accumulating more intercellular material. In 1948, Boivin et al. published figures for the DNA content of the cell nuclei of various vertebrates. They established that the DNA content of the diploid cells within a given species is constant. This was confirmed by Mirsky and Ris (1949), and by Thomson et al. (1953) in the rat. Thomson found that such factors as age, strain, sex, body weight, fasting, a protein free diet, a thiamin deficient diet and a high fat diet did not affect the DNA content of rat nuclei. In 1962, Enesco and Leblond utilized the constancy of DNA to measure cell proliferation during the growth of various organs and tissues in the young male rat. Total organ or tissue DNA was divided by the constant, 6.2 pg. (Enesco, 1957) in order to determine the number of nuclei. The total weight of the organ was divided by the number of nuclei in order to determine the amount of material associated with each nucleus. This gave a rough estimate of cell size, although it included intercellular material. In 1965, Winick and Noble determined cell number and cell size in the tissues and organs

of the rat *in utero* and postnatally. Weight/DNA and protein/DNA ratios were used as an expression of cell size, and the RNA/DNA ratio as an index of protein synthesis. Later the authors expressed results as total DNA, RNA, and protein when reporting the effects of underfeeding (Winick and Noble, 1966) and overfeeding (Winick and Noble, 1967), on the cellularity of the rat.

The measurement of cell number and cell size has added a valuable dimension to the study of kidney growth. Calculating weight/DNA, protein/DNA, RNA/DNA and lipid/DNA ratios gives an approximation of the average size and composition of the cells. Some cells are more highly differentiated than others, however, and have more protein, RNA or lipid associated with them. The figure representing cell number is also somewhat arbitrary. The chemical methods for measuring DNA do not differentiate between cells of the parenchyma and those of such fractions as blood vessels, nerves and connective tissue. It is known that the parenchymal cells of an organ make up the majority of the cells, and the connective tissue cells almost all of the rest (Enesco and Leblond, 1962). In reference to liver, Munro and Fleck (1969) have concluded that most of its mass and most of its DNA content can be attributed to hepatocytes and only a "tolerably low error" is introduced from the presence of other cells. Enesco and Puddy (1964) showed that 60 to 70% of the nuclei of muscle fibers in rat, or 85% of muscle mass was attributable to the contractile cells. Control group mice in the simulated high altitude experiments of Naeye (1966) were shown to have the following approximate percentages of parenchymal cells in selected organs: heart, 87%; liver, 78%; and adrenal cortex, 83%.

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Growth as Expressed by an Increase in Weight

Growth may be defined simply as an increase in size or weight. Organ and tissue weight increase progressively from early prenatal life until maturity. During this time the chemical composition of the organs and tissues is not static, but is changing in a manner which leads to chemical and functional maturity. Rahill and Subramanian (1973) have stressed that the fetal kidney is characteristically different from the postnatal kidney in that it has a high water content and thrives in an hypoxic environment. Widdowson and Dickerson (1960) found that in kidney, as well as in other soft tissues, there is a proportionate decrease in water and an increase in the proportion of nitrogen from early gestation until adulthood. Fetal kidney, heart and liver were found to reach their adult chemical composition before skin and skeletal muscle, and it was suggested that this is related to their earlier functional development.

Each organ and tissue contributes a certain proportion to the body as a whole and the proportion is not the same at all ages (Widdowson and Dickerson, 1960). In general, kidney weight in relation to body weight decreases from birth to maturity, unlike an organ such as the liver in which the relationship remains relatively constant. Widdowson and Dickerson (1960) found human kidneys to form a higher proportion of body weight at birth than at 13 to 14 or 20 to 22 weeks of gestation, and in adulthood. In the pig, the kidneys contributed most to the total weight of the body at 46 days of gestation. The weight of the kidneys of the newborn infant, relative to body weight, is three times greater than in the adult (Allen, 1951). The kidneys of the adult comprise about 0.4% of the total body weight (Pitts, 1968).

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Solomon and Bengele (1973) state that the relationship between organ weight and body weight as a function of development has received little attention, with very few reports referring to the kidney. The majority of the studies of the kidney show that following birth there is an increase in the ratio to a peak level, followed by a decline (Jackson, 1913; Kittelson, 1917; Arataki, 1926). In the mature animal, the ratio remains constant. Solomon and Bengele (1973) suggest that the organ weight to body weight ratios for kidney, heart, liver and spleen peak at different times, experience a different rate of decline, and are not determined exclusively by a common body weight factor such as amount of fat or change in skeletal or muscle mass. Each of these organs has its own intrinsic growth pattern relative to body weight.

In 1913, Jackson showed that the kidney weight to body weight ratio of the albino rat increased from 0.96 at birth to a maximum of 1.44 at twenty days. The ratio decreased thereafter to 1.03 at ten weeks and 0.93 at five months. Kittelson (1917) attributed this early, rapid increase in the ratio, as well as its subsequent decline, to the growth of the medulla. He found that the growth index of the kidney cortex remains quite constant and corresponds to that of the body as a whole, while the medulla shows a more varied relative growth rate. The medulla was found to double its growth index (relative to body weight) between birth and two to three weeks of age. Thereafter it showed a continuous, marked decrease, indicating that its growth lagged behind that of the whole body. The volumetric ratio of medulla to cortex was found to vary in the following manner: 1:3.05 at birth; 1:2.02 at at two weeks; 1:1.50 at three weeks; 1:2.10 at twelve weeks and 1:2.87 in adulthood.

Growth as Expressed by an Increase in Cell Number and/or Cell Size

Enesco and Leblond (1962) were the first to explore the relative contribution of cell division and cell enlargement to growth. In a comprehensive study of the male Sherman rat, they assessed DNA and weight/nucleus of whole body, tissues and selected organs including kidney, lung, pancreas, adrenal, testes, thymus and liver. The rats were sacrificed and examined at 0, 7, 17, 34, 48 and 95 days of age. Growth of the organs and tissues, in general, was characterized by three phases: a) from birth until 17 days of age, growth occurred almost exclusively by cell division, b) between 17 and 34 to 48 days of age, cell division proceeded at a reduced rate while cell size increased, especially in the tissues, c) after 48 days of age, the addition of new cells slowed down. The cells of such tissue as muscle and adipose continued to enlarge whereas in the organs there was no further increase in cell size. In regard to the kidneys specifically, the number of cells were found to increase rapidly after birth and then more slowly until a constant rate of cell division was reached. There was no change in weight/nucleus, or cell size, between 7 and 17 days. There was however, a rapid increase in weight/nucleus between 17 and 48 days. After this time, cell size remained constant. Between 7 and 95 days of age, the number of nuclei in the kidneys of the rats increased 6.5 times while the weight/nucleus doubled. (Enesco and Leblond, 1962; Enesco, 1956).

Winick and Noble (1965) confirmed the finding of Enesco and Leblond that cell division plays a major role in the growth of organs. The same conclusion had been drawn earlier by Kurnick (1951) in reference to the kidneys. Winick and Noble expanded their study of the cellular growth

of the rat to include the gestational period where they found growth to occur only by cell division. It was found that from 10 days after conception until 13 days postnatally growth is characterized by rapid cell division. Following this time, the rate of increase declines at different times in different organs. As assessed by thymidine - ^{14}C incorporation, DNA synthesis was found to have ended by the forty-fourth day postnatally in the kidneys, and had been the highest at four days.

Regarding cell size, Winick and Noble (1965) plotted weight/DNA and protein/DNA ratios for whole rat and individual organs. A discrepancy existed between the ratios during the neonatal period, when the weight/DNA ratios decreased and the protein/DNA ratios either remained constant or increased. This was attributed to water loss during the neonatal period. The authors found that there is a steady increase in organ protein, beyond the cessation of DNA synthesis, until a steady state is reached between protein synthesis and degradation at which time growth is complete. In a fashion similar to Enesco and Leblond, they have classified the growth of whole body and individual organs from ten days after conception until maturity into three phases:

a) Growth by rapid cell division

b) Growth by cell division and cell hypertrophy

c) Growth by hypertrophy alone.

The cellular growth pattern of the kidneys has been fairly well defined, and the literature (Kurnick, 1951; Zumoff and Pachter, 1964; Widdowson et al., 1972; Potter et al., 1969) has supported and expanded the findings of Enesco and Leblond (1962) and Winick and Noble (1965). By total nuclear count, Zumoff and Pachter (1964) verified that there is a diminution of nuclear division in the kidney during the mid portion

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of its active growth phase. They suggest that the sex hormones play a role in inhibiting cell division at this time. Potter et al. (1969) found that more than half of the increase in kidney weight with growth is reflected by an increase in cell number, while increasing cell size plays its major role after the rat has attained a body weight of 200 g.

Priestley and Malt (1968) traced changes in nucleic acid and protein synthesis in mouse kidney from early gestation (16 days after mating of parents) until adulthood. They found that the concentrations of DNA and RNA reached a peak within the first week of life, declined to a stable level by 40 days and persisted at this level until 200 days of age.

In regard to protein synthesis, Priestley and Malt found the rate to be twenty times as great in the fetal kidney as in the adult kidney. By five to six days after birth the rate of synthesis had decreased to twice that of the adult kidney. The authors found that along with a sharp decrease in kidney fluid just before birth, there was more of an increase in protein than could be accounted for by the change in dry weight. They attributed the greater content of kidney protein at this time to a high rate of fetal protein synthesis. This speculation is in contrast to that of McCrory (1972) and Winick and Noble (1965) who attribute the increase to alterations in body fluid volume compartments relative to the birth process. Priestley and Malt (1968) found that from five to six days after birth the total protein content of the kidney increased slowly and gradually to a plateau at 45 days of age. There was no appreciable decrease in the rate of protein synthesis up to 200 days. This adds credence to the view of Winick and Noble (1965) that the experimentally observed increase in the protein/DNA ratio with

age is not a consequence of increased rates of protein synthesis but of decreased rates of cell division.

The cellular pattern of growth in rat and mouse kidney then, is similar. Studies of the cellular growth pattern of human kidneys are limited. By examining the kidneys, heart, gastrocnemii and liver of human fetuses and newborn infants Widdowson et al. (1972) observed two distinct phases of prenatal organ growth. One was a period of rapid cell division between the earliest observed week (week 13) and the 25th week, when total organ DNA doubled weekly. The other period was characterized by a rapid increase in the protein/DNA ratio and was observed during the last 10 weeks of intrauterine life. Although, the cells were still dividing during the last ten weeks, the organs had only 20% of the adult number of cells at birth. By birth, the cells of the kidneys had nearly achieved their final size. Winick et al. (1972) concur that cell division increases in human fetal organs from 13 to 14 weeks of gestation through term, and into the first year of life. They maintain, however, that cell size in the kidney remains unchanged throughout gestation. There is a discrepancy as to this finding and that of Widdowson et al. (1972). Winick and Noble suggest that in the kidney, there is no change in cell size until after the first year of life.

The Accumulation of Protein and Lipid

An organ increases in size due to the accretion of protein, water, lipids and nucleic acids. Most of the protein is packaged within cells, and that outside the cells is primarily collagen. The lipids of the kidney are mainly triglyceride and the phospholipids and cholesterol of membranes. Lipid, in the form of osmiophilic droplets, is evident in

the interstitial cells of the cortex and especially in those of the medulla (Osvaldo and Latta, 1966; Moffat, 1975).

Protein and Differentiation

The development of an organ involves not only growth in size or mass, but includes the process of differentiation. Differentiation involves primarily the synthesis of specific protein molecules, and refers to the production of the stable, structural elements of a cell (Goss, 1964). The process leads to diversification of cell structure and function, and in the kidney results in the development of at least sixteen different cell types (Nowinski and Goss, 1969).

Nephron induction is complete by three or four weeks after birth in the rat (Kittelson, 1917; Arataki, 1926; Boss et al., 1963; Bengele and Solomon, 1974) and, in the human, by the time the fetus weighs between 2100 and 2500 g. (Potter and Thierstein, 1943). Differentiation of the cells then continues, and the nephrons change in size and configuration as they assume proper positioning in the kidneys.

Each segment of the nephron is lined with a specific type of epithelium. Bowman's Capsule is a double walled cup of squamous epithelium, the visceral layer of which becomes modified as it comes into contact with the glomerulus. At this junction, specialized cells called podocytes develop and send processes which surround the capillaries. The squamous epithelium of Bowman's Capsule changes to cuboidal epithelium at the neck of the proximal tubule. This tubule includes microvilli, and a brush border which McCrory (1972) considers to be a structurally integrated subcellular organele, not unlike the mitochondria. The brush border ends at the Loop of Henle where squamous

epithelium again becomes apparent. The epithelium continues throughout the distal and collecting tubules where it becomes more cuboidal and then columnar (Bloom and Fawcett, 1975).

Types of cells which are found in the glomerulus are endothelial cells, mesangial cells, and smooth muscle cells along with the previously mentioned podocytes. The basement membrane contains collagen as measured by hydroxproline analysis (Goodman et al., 1955; Bonting et al., 1961; Latta, 1961), and in man increases in thickness from birth to three years of age.

Other cell types in the kidney include the specialized cells of the juxtaglomerular apparatus, and the fibroblasts, chondrocytes and osteoblasts of connective tissue.

Kidney Lipids

Several investigators have attempted to define the lipid composition of kidneys, and in particular, to establish whether there is a divergence in the lipid content of the cortical and medullary zones. Morgan et al. (1963), Gold (1970) and Muller et al. (1976) have found the phospholipid content of the cortex to be greater than that of the medulla in the rabbit, dog and rat, respectively. In the same studies, triglyceride and cholesterol were found to a greater extent in medulla than cortex in rabbit and dog kidney while the opposite was true for the rat. Muller et al. (1976) found a corticomedullary concentration gradient (cortex, outer medulla, inner medulla) in adult rat kidney for total lipids, cholesterol, phospholipids and free fatty acids.

There appears to be little zonal difference in the fatty acid composition of the various lipid fractions of the kidney (Muller et al.,

1976). Several investigators (Gold, 1970; Muller et al., 1976) have found a high concentration of arachidonic acid in the phospholipids of kidney. This is probably related to its role in the synthesis of prostaglandins.

Factors Which Affect Renal Growth

Growth of the kidneys is limited at the histological level by the number of nephrons which are induced. Although no new nephrons are produced after the kidney matures, the organ is capable of adjusting its size in response to an increased work load. In this context, renal growth may be stimulated by such experimental methods as partial nephrectomy, nephrectomy serum, ureteral ligation, protein and folic acid injection, diets high in protein or sodium and low in potassium, NH_4Cl acidosis, thyroxine, testosterone, cold exposure, growth hormones, and mineralocorticoids (Nowinski and Goss, 1969).

During early development, before differentiation is complete, several factors are known to influence cell size, and cell and glomerular number. Naeye (1966) found subnormal kidney weights in undernourished mice, and in mice raised in an hypoxic environment. Growth retardation took different forms with each stress. The kidneys of the hypoxic mice were small because they had a deficient number of cells, while both cell number and cell size were affected in the undernourished mice. In both groups there were significantly fewer glomeruli than in the controls. Zeman (1966, 1967, 1970) and Zeman and Stanbrough (1969) found that the offspring of undernourished rats showed a reduction in kidney weight (relative to body weight), fewer nephrons, decreased kidney DNA, RNA, and protein and immature renal function. Accordingly, Winick and Noble (1966) established that caloric restriction during the suckling

period results in retarded organ growth as represented by a decrease in cell number. Having ascertained that undernutrition early in development influences renal growth, attention has been focused on the effect that accelerated growth might have on renal weight and cellularity.

Accelerated Growth, Obesity and Renal Development

Experimental Methods of Accelerating Growth

With the current interest in obesity being centered on prevention, numerous studies have investigated the influence of early infant feeding practices on the accumulation of excess body fat. Several research models have been devised for studying overnutrition in the suckling rat. This period in life is critical in determining the subsequent weight and food intake pattern of the animal (Kennedy, 1957a, 1957b; Widdowson and Kennedy, 1962; Widdowson and McCance, 1960).

Litter Size Reduction

The redistribution of newly born mammals into smaller litters has proven to be an effective means of overfeeding. (In theory, each pup then receives a greater proportion of the dam's milk, and is able to more fully "achieve its growth potential.") Normally rats are born in litters of eight to twelve. Reducing the litter to three should provide a model of overnutrition, while raising the animals eighteen per litter would have the opposite effect. Using these extremes in litter size, Widdowson and McCance (1960) found that rats raised in small litters weighed two to four times as much at weaning as did those from large litters, and that the former became much larger adults.

Parks (1926) was one of the first to observed the effect of litter

size on the growth of rodents, when he traced growth curves of mice born in litters of one to ten. He found that mice born three, four and five per litter showed better growth than those born in litters of six through ten, and concluded that growth was inversely proportionate to the size of the litter. Heggeness et al. (1961) has also assumed that rate of growth is inversely related to litter size, due to the availability of milk. In a study by Winick and Noble (1967), however, rats raised three per litter grew no differently than those raised six per litter, although the three per litter and six per litter groups did weigh significantly more at weaning and in adulthood than the twelve per litter controls. In contrast, Wurtman and Miller (1976a) were unable to produce accelerated weight gain and lipid storage of a persistent nature in rats raised in litters under twelve (2, 4 or 8 per litter).

Supplemental Feeding

A method of overfeeding in which the quantity and quality of milk can be modified during the suckling period is supplemental force feeding. The two procedures which have been employed include repeated tube feeding and gastrostomy with continuous infusion of liquid (Winick, et al., 1972).

Repeated tube feeding has been carried out successfully either as a complete substitute for dam delivered milk (Dymsza et al., 1964; Miller and Dymsza, 1963) or as supplemental feedings (Czajka-Narins and Hirsch, 1974; Wurtman and Miller, 1976b). The technique has proven to be time consuming and technically difficult. Particular attention must be given to the osmolarity (Aprille and Rulfs, 1976) and particle size of the supplement. The pups sometimes regurgitate or aspirate the

feeding into their lungs (Czajka-Narins and Hirsch, 1974). The sucking reflex is apparently essential for proper gastric emptying of the load (Miller and Dymsza, 1963).

Bull and Pitts (1971) have found that force feeding a supplement exceeding 35% of stomach capacity does not cause alterations in the absorption of dietary energy. With this in mind, Czajka-Narins and Hirsch (1974) developed a model for overfeeding suckling rats, in which a supplemental nonfat dried skim milk formula was delivered four times daily by bulbous tipped syringe. The supplemented animals showed accelerated growth with significantly more body fat at an early age (14 to 20 days), and more body protein throughout the 105 days of the study. Somewhat different results were attained by Wurtman and Miller (1976b) when they supplemented suckling rats with Dymsza's (1964) formula, and with commercial baby food. The experimental animals in this study had persistently more body fat from the seventeenth to the sixtieth day than did their non-supplemented controls, but carcass protein was not different. In the former study, supplementation was extended from the first to the twenty-first day of life, while in the latter the rats received feedings from the tenth to the sixteenth day.

Feeding a High Fat Diet

Obesity has been produced experimentally by feeding a high fat diet to weanling rats. Greater weight gains occur when Crisco and lard furnish the calories than when vegetable oils are fed (Barboriak, et al., 1957). In 1955, Mickelsen et al. gave Osborne-Mendel rats a diet, ad libitum, in which 85% of the calories came from Crisco. Seventy percent of the experimental male rats attained weights close

to, or over 1,000 g. Similar results were achieved by Barboriak, et al. (1957) when he fed rats a diet in which 81% of the calories came from lard.

Fenton and Carr (1951) noted a strain difference in the rate of fat deposition when four strains of mice were fed a high fat diet. Exploring the response of rats to nutritionally induced obesity, Schemmel et al. (1970) reported that the Osborne-Mendel strain responded maximally to high fat feeding, while S 5B/P1 rats showed minimal response. Strain and ration were equally influential in the development of obesity with strain most influencing body weight, and diet the accumulation of body fat. Peckham et al. (1962) also found the latter to be true.

Accelerated growth may be implemented in the suckling rat by feeding a high fat ration to the lactating dam. Emery et al. (1971) and Schemmel et al. (1973) have shown that this dietary regimen increases the caloric density of the milk. Dams fed a high fat diet secrete milk containing almost twice as much fat as that of grain-fed dams. In Emery's study, overfed pups from the Sprague Dawley strain showed accelerated weight gains at two weeks of age. Schemmel et al., using the obesity-susceptible Osborne-Mendel strain, produced overfed rats which by ten days of age were 50% heavier than the controls. In both studies, weight gain and the accumulation of body fat by the time of weaning were significantly greater in those animals that had received milk from high fat-fed dams than in those whose dams had been fed grain. The experimental Osborne-Mendel rats had almost four times as much body fat as did the controls.

Accelerated Growth and Renal Development

One of the most prominent manifestations of obesity is cardiovascular renal disease. Weil pointed out in 1955, that excessive perirenal fat surrounding the kidneys could be detrimental to their function. Kennedy (1957c) found that kidney lesions, similar to those of advancing age, were apparent earlier in rats made obese by hypothalamic lesion. Renal damage to the degree which caused death in rats over two years of age was evident in obese rats from one year to fifteen months of age. Accordingly, Johnson (1972) found significantly more perirenal fat, a greater number of kidney lesions and lesions of a more severe nature in 45 week old Osborne-Mendel rats made obese by feeding a high fat diet. Aprille and Rulfs (1976) also described large storage depots of fat around the kidneys of ten day old rats that had been tube-fed a 78% fat diet. Widdowson and Kennedy (1962) found an increased incidence of kidney disease in fast growing (3/litter vs. 15-20 litter) male rats, whereas Barboriak (1957) found no systematic influence of an 81% fat or oil diet on kidney damage up to two years of age.

From comprehensive studies on growth and development (Widdowson and McCance, 1960; McCance, 1962; McCance and Widdowson, 1962; Widdowson and Kennedy, 1962) have come to the following observations:

1. During normal growth, all parts of the body do not develop equally fast or at the same time.
2. Undernutrition alters the shape and form of the growing animal because those parts which develop before others retain priority of growth and may continue to increase in size at the expense of others (undernutrition affects growth of the skeleton less than it does growth of the adipose).

3. A very high plane of nutrition, by saturating the growth requirements of the animal, accelerates the development of all tissues, but particularly those with little structural stability and low priority (i.e., adipose tissue).

Accelerating body weight gains early in development results in a proportionate increase in the size of the kidneys. Thus, kidney growth is related to body size rather than to age (Moment, 1933; Widdowson and McCance, 1960; Rakusan, 1975). The young overfed rats in experiments by Solomon and Capek (1972a) and Solomon and Bengele (1973) had lower kidney weight/body weight ratios than did the controls, regardless of differences in body fat. The authors surmized that the kidney weight/body weight ratio in these animals had already peaked and was on the decline, indicating greater organ maturity. Tests as to functional development substantiated this theory (Solomon and Capek, 1972b).

Winick and Noble (1967) studied the cellular aspects of accelerated growth in the organs of neonatal rats. Raising animals three and six per litter resulted in kidneys which weighed 14% more at weaning than did those of the controls. Through this study it was established that increasing calories during the suckling period accelerates organ growth exclusively by increasing the rate of cell division.

INTRODUCTION

In 1970, Schemmel et al. reported a difference among seven strains of rats in their susceptibility toward obesity when fed a high fat (60% w/w) diet from the time of weaning. The Osborne-Mendel rats responded by accumulating up to 50% body weight as fat, while the S 5B/Pl rats maintained only 14% fat in their bodies. As a result of this study there was interest as to how these two strains of rats would respond to aggressive overfeeding earlier than 21 days of age. Introducing more calories during the suckling period might simulate the human situation where infants in our society are often exposed to calorically dense foods earlier in life than is necessary. The overall experiment was designed to study the effects of overnutrition during the suckling period on the growth and development of whole body and individual organs and tissues in two strains of rats. Three overfeeding techniques were utilized in an attempt, particularly, to force the development of obesity in the S 5B/Pl animal. These techniques included a) feeding a high fat (44% w/w) diet b) supplemental force-feeding and c) litter size reduction.

In 1972, Johnson used the Osborne-Mendel rat and an experimental 60% fat (w/w) diet in studying kidney development and function in the obese animal. She reported total kidney weights to be significantly greater at 25, 35, and 45 weeks of age in those Osborne-Mendel rats fed the high fat diet from weaning than in those fed a grain ration.

The purpose of the present investigation was to ascertain whether early overfeeding practices would cause an increase in the weight of the kidneys of obesity-susceptible (Osborne-Mendel) and obesity-resistant (S 5B/Pl) rats. If such an increase in weight occurred, an attempt would be made to determine whether it was due to an increase in cell number, cell size or both. Observation would also be made of the changes in the number and size of the cells between 24 and 105 days of age.

METHODOLOGY

Conditions of the Experiment

Eighty newborn, male rats from the S 5B/Pl[1] strain and eighty from the Osborne-Mendel strain[2] were assigned to the experimental design depicted in Table 1. The rats were organized into litters of three or six, and were assigned either to receive, or not to receive supplemental force-feeding. A high fat diet was fed to one-half of the dams in each strain from partuition until the pups were weaned. The other half received a low fat diet. One-half of the suckling rats from each strain was raised to weaning (24 days) and the other half to 105 days of age. Upon weaning, those rats not sacrificed were maintained on the respective high or low fat diet of their dams until 105 days. The variables in this experiment then, were Age, Strain, Diet, Supplementation and Litter Size. There were 32 different treatments, with 5 rats in each category.

The composition of the high and low fat diets that were fed to the dams and weaned pups is shown in Table 2. Forty-four percent

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[1]The pedigreed S 5B/Pl Cr breeding stock was secured through the generosity of Samuel L. Poiley, former Head, Mammalian Genetics and Animal Production Section D R and D, Chemotherapy, National Cancer Institute, DHEW.

[2]Originally, ninety-six rats from each strain were included in the study. For statistical purposes, due to the death of several rats, the number was randomly cut to 80 per strain; 5 rats per treatment, rather than 6.

Crisco, by weight, was used in the high fat ration, comprising 70% of the total calories. Seven percent of the calories in the low fat ration were from fat, in the form of corn oil. Carbohydrate, as cerelose, contributed 7% of the calories in the high fat diet, and 70% in the low fat ration. In both rations, 23% of the calories came from protein, as casein. Each gram of the high fat ration contained 5.67 kcal., and each gram of the low fat ration, 3.81 kcal. In both rations there were 17.2 kcal/g. protein. The rations and drinking water were available ad libitum.

The supplementary feeding formula simulated the fat content of milk produced by auxillary dams of the same strains fed high and low fat diets. The milk fat was analyzed by the Roese-Gottlieb Method as outlined by the Association of Official Analytical Chemists (Horwitz, 1970).

The mean fat content of milk from S 5B/Pl dams fed the low fat ration was 15%, while that from dams fed the high fat ration was 20%. Respective percentages in the milk of Osborne-Mendel dams were 18 and 24%. This was based on the analysis for fat of three to five samples for each group of rats. Four supplemental milk mixtures were prepared incorporating corn oil in the above percentages. Fifteen, 20, 18, and 24 ml. of corn oil plus, in each case, 15 g. of nonfat dry milk solids were made to volumes of 100 ml. with distilled water. The mixtures were shaken well before use. The method for force-feeding the pups was that of Czajka-Narins and Hirsch (1974). The pups were force-fed between 8:00 and 9:00 a.m. and 4:00 and 5:00 p.m. each day from birth until weaning. The nonsupplemented pups were handled each day in like manner, but not force-fed.

The experimental animals were housed in wire screen cages (18x18x25 cm.). A temperature of 23±1 degree centigrade was maintained in the room, and conditions were such that each 12 hour period of light was followed by 12 hours of darkness. Disturbances were kept at a minimum during cage cleaning, feeding and handling.

Sample Preparation

Upon sacrifice, the animals were weighed, anesthesized with ether, and decapitated. The kidneys of each animal were quickly removed, decapsulated, placed on aluminum foil and set on dry ice. Then the organ was weighed, wrapped in aluminum foil, placed in a Zip-lock plastic bag[3] on dry ice and later stored in a freezer at -18 degrees centigrade.

The day before each organ in a series of samples was ground in preparation for DNA, protein and triglyceride analysis, the amount of distilled deionized[4] water which would dilute the kidneys to a volume ten times their weight (w/v) was calculated:

$$\text{Kidney weight at sacrifice (g.)} \times 10 - \text{Kidney weight at sacrifice (g.)} = \text{Water to dilute}$$

This amount of water was measured by pipette, and equally divided between two labelled 55 ml. test tubes. The tubes for each sample were capped with parafilm[5] and refrigerated overnight at 0 degrees centigrade. For each sample, 5 ml. of 10% trichloroacetic acid, 10 g./100ml. (w/v), was

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[3]Lab Apparatus Company, 18901 Cranwood Parkway, Cleveland, Ohio.

[4]Illco-Way Ion X Changer, Research Model I, Illinois Water Treatment Co., 840 Cedar Street, Rockford, Ill.

[5]American Can Company, Neenah, Wisconsin.

pipetted into a centrifuge tube in preparation for protein analysis. In similar fashion, 5 ml. of 20% trichloroacetic acid, 20 g./100ml. (w/v), was pipetted into a centrifuge tube for DNA analysis. All of the tubes were then capped with marbles and refrigerated overnight.

On the day of sample preparation, the tubes containing water, and those containing 10 and 20% trichloroacetic acid were taken from the refrigerator and placed in a bed of crushed ice. The samples to be ground were taken from the freezer and placed on dry ice. As each organ was processed, it was unwrapped and placed in the first tube of premeasured cold water. The tube with water and sample was placed in a 400 ml. beaker filled with crushed ice. The sample was then homogenized at speeds 5 and 6 of a Brinkman polytron[6] for a time necessary to insure complete dispersion of the sample. Tweezers were used as needed for removing fibers which had caught in the blades. The tube was then removed and placed on ice, and the second tube of water was used to rinse the blade of the polytron. This portion was then added to that of the first tube. The blade of the polytron was then rinsed with distilled deionized water, ethyl alcohol and acetone, in preparation for the next sample.

While swirling the contents of the ice-clad tube, 1 ml. of homogenate was transferred by pipette into the chilled centrifuge tubes for protein analysis (10% TCA). For DNA analysis, the amount of homogenate that was transferred to iced tubes (20% TCA) was 0.5 ml. for the 24 day old rats and 1.0 ml. for the 105 day old rats. The remainder of the homogenate was topped with parafilm and stored in the freezer until

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[6]Brinkman Instruments Inc., Cantiague Rd., Westbury, N.Y.

triglyceride analysis could be performed. The contents of the centrifuge tubes were mixed on a Vortex test tube mixer[7] for several minutes. The tubes were then placed in a Sorval Model RC2B refrigerated centrifuge[8] and spun at -15 degrees centigrade and 3,000 r.p.m. for twenty minutes. At the end of this time, the supernatant was decanted and discarded from both protein and DNA tubes. The tubes containing the portion for protein analysis were capped with marbles and refrigerated at 0 degrees centigrade for analysis within the week. The samples for DNA assay were processed immediately.

DNA Analysis

The method which was employed for DNA analysis was Burton's Modification of the Diphenylamine Reaction (Burton, 1956). This reaction is between the deoxypentose of DNA and diphenylamine in a mixture of glacial acidic acid and sulfuric acid. Acetaldehyde potentiates the color development. The solutions that were used in the analysis were prepared as outlined in the Appendix.

To the pellets which remained on ice in the centrifuge tubes (20% TCA), 6 ml. of room temperature, 5% trichloroacetic acid, 5g./100 ml. (w/v), were added by pipette. The samples were placed in a hot water bath[9] at 90 degrees centigrade for 30 minutes. Any precipitate adhering to the sides of the tubes was scraped down by stirring rod into the TCA solution. The samples were then cooled on

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[7]Model K-500-2, Scientific Industries Inc., Springfield, Mass.

[8]Ivan Sorvall Inc., Newtown, Conn.

[9]Precision Scientific Co., Chicago, Ill.

ice, and centrifuged at 3,000 r.p.m. and -15 degrees centigrade, for 10 minutes.

Two milliliter duplicates of the supernatant of each DNA sample were transferred by pipette into labelled 25 ml. test tubes. To the tubes were added by pipette, 4 ml. of freshly made diphenylamine-acetaldehyde solution (Appendix). The contents of the tubes were mixed on the Vortex test tube mixer, capped with glass marbles and allowed to stand overnight (16-20 hours) at room temperature.

Two sets of standards were treated similarly. The standard was calf thymus deoxyribonucleic acid,[10] and the solution was made as described in the Appendix. Appropriate increments of the standard were diluted with 5% trichloroacetic acid in order to attain a volume of 2 ml., and concentrations of 0, 10, 20, 30, 70 and 100 ug/ml. DNA. The standards were treated with 4 ml. of the diphenylamine-acetaldehyde solution, mixed, capped with glass marbles and allowed to stand overnight along with the treated samples.

The next day, absorbance of the samples and standards was read on a Beckman, Model DB spectrophotometer[11] at 700 and 595 nm. The blank was 2 ml. of 5% trichloroacetic acid which had been treated along with the standards. If any reading was observed at 700 nm., it was subtracted from the absorbance at 595 nm. There was found to be little, if any, interference at 700 nm. Duplicates of the samples agreed within 5%. Absorptivity was calculated for each concentration

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[10]Type 1, D 1501, Sigma Chemical Corporation, P.O. Box 4508 St. Louis, Mo.

[11]Beckman Instruments Inc., 2500 Harbor Blvd., Fullerton, Cal.

of the standard. When the mean absorptivity of one set of standards was not the same as the other, the value of the set which gave the best standard curve was used. The mean absorptivity was used to calculate the amount of DNA in the kidneys as follows:

$$\frac{\text{Absorbance, unknown}}{\text{Mean absorptivity}} = \text{Concentration DNA, unknown}$$

$$\frac{\text{Concentration DNA, unknown}}{\text{Aliquot size (supernatant)}} \times \frac{\text{Total volume 5\% TCA}}{\text{Sample size (homogenate)}} \times \text{Total volume of homogenate}$$

Protein Analysis

Total kidney protein was assessed by the method of Lowry (1951). The reaction is between Folin-Ciocalteau reagent (molybdate, tungstate, phosphoric acid) and the proteins, tryptophan and tyrosine. Cupric ion intensifies the development of the blue color. The solutions used in the analysis were prepared as outlined in the Appendix.

To the previously refrigerated centrifuge tubes containing the pellets (10% TCA) for protein analysis was added 5 ml. of 1N NaOH.[12] The contents of each tube was mixed on the Vortex mixer until all of the pellet was in solution.

Into a series of 55 ml. test tubes was pipetted 0.95 ml. of distilled deionized water. After thoroughly re-mixing the samples, 0.05 ml. duplicate aliquots were removed by pipette and added to the tubes containing the water. The contents of all of the tubes were then thoroughly mixed. Using the same Vortex mixer, 5 ml. of copper sulfate-sodium carbonate-sodium tartrate solution (Appendix) was added by

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[12]USP, J. T. Baker Chemical Co., Phillipsburg, N.J.

pipette to each constantly agitated tube. After ten minutes and as each tube was constantly agitated, 0.5 ml. of 1N Folin-Ciocalteau[13] solution (Appendix) was added to each tube by micropipette. The samples were allowed to sit at room temperature for 30 minutes, after which absorbance was read at 740 nm. on the Beckman DB spectrophotometer.

Two sets of standards were run with the samples. The standard was crystalline bovine albumin,[14] and it was prepared as outlined in the Appendix. Just before use, 1 ml. of the standard stock solution was pipetted into a 10 ml. volumetric flask. The solution was made to volume with 1N NaOH. The contents were mixed by inverting the flask several times. Appropriate increments of the standard were combined with distilled deionized water to a volume of 1 ml. The set contained 0, 50, 100, 150, 200, 250 and 300 ug/ml. The standards were mixed and treated with the copper sulfate-sodium carbonate-sodium tartrate and Folin-Ciocalteau solutions along with the samples. After observing the absorbance at 740 nm., absorptivity was calculated for each concentration of the standards. Again, if the mean absorptivity of one set of standards was not the same as the other, the one from the set that gave the best standard curve was used. The amount of protein in the samples was calculated by the use of the mean absorptivity, in the following manner:

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[13]Phenol Reagent Solution 2N (Folin-Ciocalteau), SO-P-24, Fisher Scientific Co., Fair Lawn, N.J.

[14]No. 7285, Nutritional Biochemicals Corporation, Cleveland, Ohio.

$$\frac{\text{Absorbance, unknown}}{\text{Mean absorptivity}} = \text{Concentration protein, unknown}$$

$$\frac{\text{Concentration protein, unknown}}{\text{Aliquot size}} \times \frac{\text{Total volume NaOH}}{\text{Sample size (homogenate)}} \times \text{Total volume of homogenate}$$

Triglyceride Analysis

Triglyceride was measured by acid hydrolysis according to the method described by the Association of Official Analytical Chemists (Horwitz, 1970).

A series of Mojonnier extraction flasks,[15] housed in 150 ml. beakers, was set up on trays. Each flask and beaker was tared on a Mettler analytical balance,[16] and approximately 30 mg. of each kidney homogenate sample was added to the flask by pasteur pipette. The homogenate was subjected to constant mixing at speeds 5 and 6 of the polytron, in between sampling. The blade of the polytron was rinsed with distilled deionized water, ethyl alcohol, and acetone between samples. After all of the samples had been weighed, any homogenate adhering to the walls of the flasks was rinsed down by pipette with 10 ml. of hydrochloric acid (4+1).[17] A blank was included in the series, containing only 10 ml. of hydrochloric acid (4+1). The contents of each flask was mixed by shaking, and the flask was placed in a hot water bath[18] at 70 degrees centigrade. Duplicate samples were

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[15]Model G3, Mojonnier Brothers Co., 4601 West Ohio Street, Chicago 44, Ill.

[16]Type H 15, Mettler Instrument Corporation Hightstown, N. J.

[17]Four parts 37% HCl to one part distilled deionized water.

[18]The hot water baths were 3,000 ml. pyrex, water-filled beakers, each heated on a Corning, No. PC-35 hot plate, Corning Glass Works, Corning, N.Y.

analyzed where quantity permitted. The hot water bath was brought to a boil, and the samples and blank were timed for 30 minutes. After this time the contents of the flasks were cooled to room temperature, and distilled deionized water was added to the midpoint of the constricted lower portion of the flask. Twenty-five milliliters of anhydrous ethyl either[19] was added to each flask by graduated cylinder. The flasks were closed with No. 1 rubber stoppers which had been previously soaked in acetone. The contents of the flasks were shaken by hand for one minute.[20] Then 25 ml. of petroleum ether[21] was added, the stopper for each flask applied, and the contents shaken for another one minute. After the flasks had sat unstoppered at room temperature for 30 minutes, the top, clear layer of ether and triglyceride was decanted into tared Goldfisch extraction beakers.[22] The beakers had previously been washed in hot soapy water, rinsed in tap and distilled water and acetone, and dried in an oven[23] for at least one hour. Thirty minutes before weighing, the beakers were placed in a desicator for cooling and drying.

The extraction with ethyl and petroleum ether was repeated two more times, with intermittent shaking and standing at room temperature for 30 minutes. Before the last extraction, distilled water was added where needed in order to bring the contents to the same level at the bend of the flask. After each extraction, the decanted solvent was added

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[19]Analytic reagent, Mallinckrodt Chemical Works, St. Louis, Mo.

[20]In lieu of a centrifuge.

[21](30-60)° C, Analytic reagent, Mallinckrodt Chemical Works, St. Louis, Mo.

[22]Corning Glass Works, Corning N.J.

[23]Precision Scientific Co., Chicago, Ill.

to that of the first extraction.

The ether from the extraction was allowed to evaporate from the beakers overnight[24] under the hood. The next day the beakers were placed in an oven at 70 degrees centigrade for one hour. They were then transferred to desicators where they remained for 30 minutes. The beakers were weighed, and the total triglyceride and percent triglyceride were calculated by difference, in the following manner:

$$\text{Beaker wt.}_{\text{final}} - \text{Beaker wt.}_{\text{initial and Blank}} = \text{Wt. triglyceride, aliquot}$$

$$\frac{\text{Wt. triglyceride, aliquot}}{\text{Wt. aliquot} + 10\ \text{(dilution)}} = \%\ \text{triglyceride}$$

$$\%\ \text{triglyceride} \times \text{Kidney wt.} = \text{Total kidney triglyceride}$$

Methods of Statistical Analysis

The differences in the data brought about by the variables Age, Strain, Diet, Supplementation and Litter Size as well as interactions between those variables were analyzed by use of the five-way multivariate analysis of variance and univariate analysis of variance. These statistics along with group means and standard deviations were compiled at the Michigan State University Computer Center. There were no 5-way nor any 4-way interactions which significantly influenced the results, and the majority of 3-way interactions involved the variables Age, Strain and Diet. For this reason, the data was grouped by Age and Strain, and the differences brought about by Diet tested for significance by use of the Student T Test.

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[24]Or solvents could have been evaporated over a steam bath.

RESULTS AND DISCUSSION

Total Body Weight

Tables 3 and 4 show the mean live weights of rats from each treatment of the study at 24 and 105 days of age. There was a significant ($P<0.01$) increase in body weight with age for all groups within each strain, as recorded in Table 5. S 5B/P1 rats weighed approximately six times as much at 105 days as at 24 days, while Osborne-Mendel rats weighed approximately seven times as much. The weight gain of the Osborne-Mendel rats fed the high fat diet was greater than in those fed the low fat diet for all treatments. In the S 5B/P1 strain, however, feeding a high fat diet was responsible for a greater gain in weight in only one out of the four treatments (HF, Su, 6/L).

Total body weights of the Osborne-Mendel rats were significantly greater ($P<0.01$) than those of the S 5B/P1 rats in all similar treatments (Table 5). At 24 days, mean body weights of the S 5B/P1 rats ranged from 47 to 55 g., while those of the Osborne-Mendel strain ranged from 66 to 90 g. The range in body weights at 105 days was 259 to 329 g. for the S 5B/P1 rats, and 433 to 634 g. for the Osborne-Mendel rats. The lightest rat at 105 days of the study weighed 234 g. and was from the S 5B/P1 strain (LF/6/L, Su), while the heaviest rat was an Osborne-Mendel (HF, 6/L, Su) and weighed 721 g.

There was a significant main effect of diet ($P<0.01$) on body weight in the Osborne-Mendel strain at both 24 and 105 days of age (Figure 1).

Osborne-Mendel weanling rats that had been nursed by dams fed the high fat diet weighed 13 g. more than Osborne-Mendel offspring nursed from dams fed the low fat diet. By 105 days of age, Osborne-Mendel rats fed the high fat diet had a mean body weight 128 g. greater than those fed the low fat diet (461 vs. 589 g.). There was no significant effect of diet on body weight in the S 5B/Pl strain at either 24 or 105 days. The differences in mean body weight between S 5B/Pl rats maintained on high and low fat diets were 3 g. at 24 days and 18 g. at 105 days.

Supplementation or changing litter size from six to three had little effect on body weight (Figure 2). There was no five-way interaction of Age, Strain, Diet, Supplementation and Litter Size which significantly increased values for body weight, nor were there any significant four-way interactions (Table 5). The three-way interactions of Age, Strain and Diet, and Diet, Supplementation, and Litter Size were found to be significant. The former three-way interaction, however, was the most relevant in terms of this study (Figure 1).

Total Kidney Weight

Mean weights of the kidneys of the various groups of rats are presented in Tables 3 and 4. Kidney weights increased significantly ($P<0.01$) with age (Table 5). The weights of the kidneys of all rats at 105 days were about four times the weights at 24 days. Although the kidneys of the S 5B/Pl rats weighed less than those of the Osborne-Mendel, the increase in weight between 24 and 105 days was of the same magnitude in both strains. Feeding Osborne-Mendel rats a high fat diet led to greater gains in kidney weight with age than feeding a low fat diet in all treatments except 6/L, NS, where the weight gain was the

same. In the S 5B/P1 strain, all groups of rats fed the high fat diet experienced a greater kidney weight gain with age than did those fed the low fat diet.

There was a significant ($P<0.01$) strain difference in kidney weight (Table 5). The mean weights of the kidneys of the S 5B/P1 rats ranged from 0.48 to 0.58 g. at 24 days and 1.74 to 2.38 g. at 105 days. The range of weights in the Osborne-Mendel strain was 0.63 to 0.85 g. at 24 days and 2.51 to 3.51 g. at 105 days. For comparable groups, the kidney weights of the S 5B/P1 rats were 70-75% as great as those in the Osborne-Mendel strain.

There was a significant effect ($P<0.01$) of diet on the kidney weights of the animals in this study (Table 5 and Figure 3). Kidneys weighed significantly more in rats fed the high fat diet than in rats fed the low fat diet, regardless of age or strain. At weaning, the mean kidney weight of the S 5B/P1 rats nursed from dams fed the high fat diet was 12% greater than that of S 5B/P1 rats nursed from dams fed the low fat diet (0.56 vs. 0.50 g.). In the Osborne-Mendel strain the difference was 16% (0.78 vs. 0.67 g.). By 105 days, S 5B/P1 and Osborne-Mendel rats maintained on the high fat diet had mean kidney weights 19 and 21% greater, respectively, than those maintained on the low fat diet. The mean weights, in grams were 2.22 vs. 1.88 for the S 5B/P1 strain, and 3.19 vs. 2.61 for the Osborne-Mendel.

There were no significant main effects of supplementation or litter size on kidney weight (Table 5 and Figure 4).

Kidney Weight/Body Weight Ratio

Tables 3 and 4 list the mean kidney weight/100 grams body weight (KW/BW) ratios for rats exposed to each of the thirty-two treatments of the study. The KW/BW ratios decreased in both strains with age. This is consistent with the developmental relationship that has been observed between organ weight and body weight in which, following birth, the KW/BW ratio increases to a peak level, and then declines with age (Solomon and Bengele, 1973). At weaning, the mean KW/BW ratio for all S 5B/Pl rats was 1.04 compared to 0.94 for the Osborne-Mendel rats. At 105 days, the ratios were 0.71 and 0.56, respectively. When the kidney weights are expressed on the basis of lean body mass[1] (Tables 3 and 4), there is no difference in the relative size of the kidneys in the two strains at 24 days. Thus, greater body fat accounts for the lower KW/BW ratio in the 24 day old, Osborne-Mendel rats. There may, however, be a strain difference in kidney weight by adulthood. The kidney weight/lean body mass ratio for 105 day old S 5B/Pl rats is 0.92 compared to 0.87 for the Osborne-Mendel strain. The adult S 5B/Pl rats have relatively larger kidneys than the Osborne-Mendel rats. The greater KW/BW ratio in this strain is not entirely due to the Osborne-Mendel animals having a greater amount of body fat than the S 5B/Pl rats.

Osborne-Mendel rats maintained on the high fat diet had the same KW/BW ratios, at both ages, as did the Osborne-Mendel rats maintained on the low fat diet. This finding is in disagreement with those of other overfeeding studies (Johnson, 1972; Solomon and Bengele, 1973)

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[1]As determined by carcass weight minus ether extracted fat. Heart, brain, liver, kidneys, gastrocnemius muscle and three fat depots had been removed before lipid extraction.

and can be explained by the fact that the control diet in this study was a semi-purified low fat ration, rather than a grain ration as used by Johnson (1972) and Solomon, etc. It has been found in our laboratory that those rats maintained on a semi-purified ration show greater weight gain than those fed grain (Winnie et al., 1973). The body weights of the Osborne-Mendel control rats in this study are conspicuously greater at 24 and 105 days than are those reported by Johnson (1972). On the other hand, the kidneys are comparatively of the same weight.

The KW/BW ratio in the S 5B/P1 rats was slightly higher in the high fat-fed animals. At 24 days, the ratio was 1.07 for rats weaned from high fat dams and 1.02 for those weaned from low fat dams. At 105 days, the ratios were 0.75 and 0.68 respectively.

From the data in Tables 3 and 4, it may be seen that kidney weight relative to lean body mass was greater in the high fat-fed rats regardless of age or strain. These rats had proportionately larger kidneys than did those fed the low fat diet.

Total Kidney DNA

The mean values for kidney DNA are shown in Table 6. There was a significant ($P<0.01$) increase in DNA with age (Table 5). In both strains, kidneys contained approximately two times the amount of DNA at 105 days as at 24 days. The mean total DNA content of all S 5B/P1 rats at 24 days was 2.18 mg. and at 105 days, 4.06 mg. In the Osborne-Mendel strain, the mean total DNA content was 2.54 mg. and 5.87 mg., respectively. In all treatments, the Osborne-Mendel rats fed the high fat diet gained more kidney DNA between 24 and 105 days than did those fed the low fat diet. In only one of the four treatments (6/L, Su) in

the S 5B/P1 strain was the gain in DNA with age greater on the high fat diet.

There was a significant ($P<0.01$) main effect of strain on total kidney DNA in this study (Table 5). At 105 days, mean values for DNA ranged from 4.91 mg. to 7.25 mg. in the Osborne-Mendel strain, and 3.75 mg. to 4.31 mg. in the S 5B/P1. At 24 days, the kidneys of Osborne-Mendel rats contained 0.36 mg., or 17% more DNA than those of the S 5B/P1 rats.

There was neither a five-way interaction, nor any four-way interactions which caused significantly greater scores for DNA. There was a significant ($P<0.01$) main effect of Diet, as well as Age and Strain as discussed above.

The three-way interaction of Age, Strain and Diet significantly ($P<0.01$) affected DNA values in all but the 105 day old S 5B/P1 rats (Figure 5). The kidneys of rats that had nursed from high fat dams contained significantly more cells at weaning than those rats that had been nursed from low fat dams. This was true for both strains, although the difference was only 8% in the S 5B/P1 rats. The greater cell number in the Osborne-Mendel strain was a reflection of 2.75 mg. DNA for the high fat animals and 2.32 mg. for the low fat animals. In the S 5B/P1 strain, the mean DNA content was 2.27 mg. and 2.09 mg., respectively.

By the time the low fat, S 5B/P1 rats reached 105 days, they had demonstrated catch-up growth in regard to cell number. The rats that had been maintained on a high fat diet had 4.11 mg. DNA, and those maintained on low fat, 4.00 mg. DNA.

The Diet effect on DNA was greatest in the Osborne-Mendel strain

by 105 days. There was 27% more DNA in the kidneys of the Osborne-Mendel, high fat rats than in those of the low fat rats at this age. This is represented by mean values of 5.17 mg. DNA for Osborne-Mendel, low fat-fed rats and 6.57 mg. for those fed the high fat diet. Between 24 and 105 days of age, Osborne-Mendel rats fed the high fat diet gained 3.82 mg. kidney DNA and those fed the low fat diet, 2.85 mg. DNA.

The greatest amount of kidney DNA measured was 7.25 mg. in the Osborne-Mendel, high fat, 3/litter, nonsupplemented group. Supplementation or changing litter size from 6 to 3 rats had no significant effect on kidney DNA (Table 5 and Figure 6).

Total Kidney Protein

The protein content of the kidneys of all rats in this study increased significantly ($P<0.01$) between 24 and 105 days of age (Table 5). The gain by 105 days represented four times the kidney protein at 24 days in both strains. The kidneys of 24 day old S 5B/P1 rats contained a mean of 58 mg. protein and the 105 day old S 5B/P1, a mean of 239 mg. Values for the Osborne-Mendel rats were 81 mg. and 359 mg. respectively. In all comparable treatments, in both strains, those rats maintained on a high fat diet had greater gains in kidney protein than did those maintained on low fat. The mean gain in kidney protein for the Osborne-Mendel, low fat rats was 252 mg. while that of the Osborne-Mendel high fat was 304 mg. Gains for similar groups in the S 5B/P1 strain were 167 mg. and 196 mg., respectively.

There was a significant ($P<0.01$) main effect of strain on the protein content of the kidneys (Table 5). For all comparable treatments, higher values were observed in the Osborne-Mendel strain. At

weaning, mean total kidney protein in the S 5B/P1 rats ranged from 53 to 73 mg., while the range in the Osborne-Mendel rats was 58 to 94 mg. (Table 6). At 105 days, the respective ranges were 204 to 308 mg. and 296 to 418 mg.

The variable, Diet, significantly ($P<0.01$) affected kidney protein (Table 5). The kidneys of rats that had been weaned from, or maintained on, the high fat diet had 15 to 20% more protein than those on the low fat diet. This may be illustrated by considering the interaction of Age, Strain and Diet (Figure 7). The kidneys of the rats fed the high fat diet contained more protein than those of rats fed the low fat diet. This was significant at 24 days and at 105 days in the Osborne-Mendel strain. The significance was at the 5% level in the S 5B/P1, 24 day old rats. Although there appeared to be a significant difference between the mean protein content of the kidneys of 105 day old, S 5B/P1 rats fed high and low fat diets, the significance was contributed by only one of the four treatments (6/L, NS). Because the mean value for this group of rats is considerably out of line, for no apparent reason, the significance is questionable. The mean values which the graph represents at 24 days are: S 5B/P1, low fat, 55 mg.; S 5B/P1, high fat, 62 mg.; Osborne-Mendel, low fat, 74 mg.; Osborne-Mendel, high fat, 88 mg. The respective mean values at 105 days are: S 5B/P1, 222 mg. and 257 mg.; Osborne-Mendel, 326 mg. and 392 mg.

There was no significant main effect of Litter Size on kidney protein (Table 5 and Figure 8). The main effect which surfaced for Supplementation was the result of nonsupplemented rats having more kidney protein than supplemented. At 24 days, supplemented rats of both strains had a mean of 66 mg. of kidney protein compared to 71 mg.

for those nonsupplemented. At 105 days, the mean values were 288 and 309 mg., respectively. Obviously, supplementing the diet exerted no positive effect on kidney protein. There was no significant five-way interaction apparent, nor were there any significant four-way interactions.

Protein/100 grams Kidney Weight

Values for protein/100 grams kidney weight are presented in Table 7. At 24 days kidneys in both strains contained approximately 11% protein, and at 105 days, 12%. These percentages are lower than those reported by others (Winnick and Noble, 1966, 1967; Spector, 1956). Nowinski and Goss (1969) report kidney protein content in rats of around 14%.

Protein/DNA Ratio

The protein/DNA ratios for all groups of rats are shown in Table 8. The ratios doubled in both strains between 24 and 105 days of age. The ratio in the S 5B/P1 strain increased from a mean of 27 to 59 while the increase in the Osborne-Mendel rats was from 32 to 61. The cells in the kidneys of S 5B/P1 rats were smaller at both ages than in comparable Osborne-Mendel rats. There was no difference in the protein/DNA ratio of the low fat and high fat rats within each strain and age, indicating that the cells were of the same size. The exception, again, was in the S 5B/P1, 105 day old, high fat, nonsupplemented group where the unexpected higher protein value influenced the ratio.

Total Kidney Triglyceride

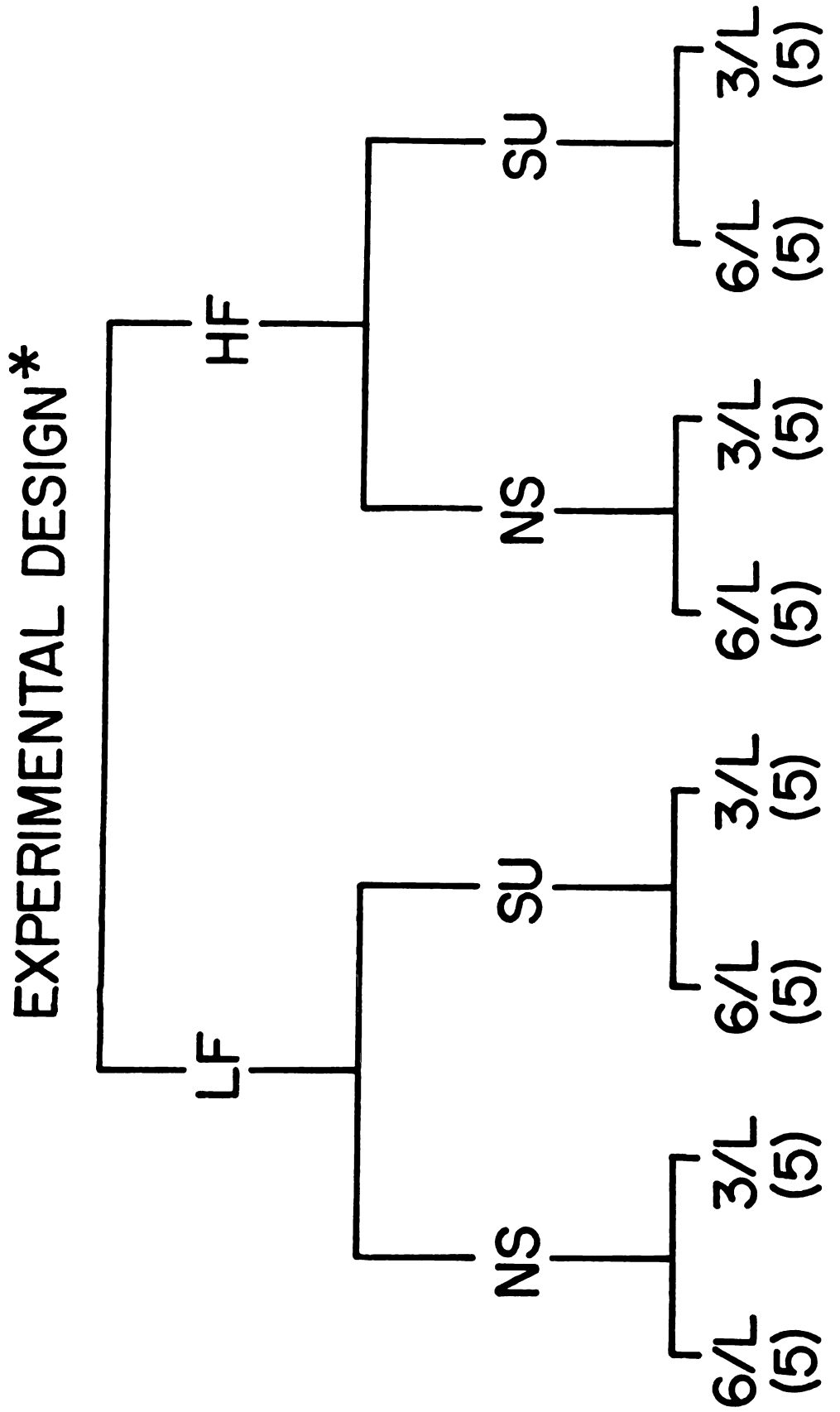
Results of the ether extraction of kidney triglyceride are presented in Table 6. The kidney triglyceride content in all groups of rats increased significantly with age ($P<0.01$), while triglyceride as a percent of kidney weight remained relatively constant at about three percent (Tables 5 and 9). The kidney triglyceride of Osborne-Mendel and S 5B/P1 rats at 105 days was three to four times that at 24 days.

There was a significant ($P<0.01$) main effect of strain on kidney triglyceride (Table 5). At 24 days of age the kidneys of S 5B/P1 rats contained a mean of 18 mg. of triglyceride, while those of the Osborne-Mendel rats contained 21 mg. By 105 days, the triglyceride content was 62 and 83 mg., respectively.

The effect of feeding a high fat diet is most clearly shown by the three-way interaction of Age, Strain and Diet (Figure 9). The triglyceride content of the kidneys of rats fed the high fat diet was significantly greater than in those fed the low fat diet in both strains at 24 days, and in the S 5B/P1 strain at 105 days of age. At weaning, kidneys of S 5B/P1 rats that had suckled from high fat dams had a mean triglyceride content of 21 mg. compared to 15 mg. for those suckled from low fat dams. At this age, in the Osborne-Mendel strain, the values were 24 mg. and 17 mg., respectively. By 105 days, mean triglyceride in the kidneys of S 5B/P1 rats fed the high fat diet was 70 mg. in contrast to 55 mg. in those rats fed the low fat diet. The kidneys of 105 day old Osborne-Mendel rats contained 88 mg. and 78 mg. for rats fed high and low fat diets, respectively. Although the difference in triglyceride between the two diets was significant for both strains at weaning and for the S 5B/P1 rats at 105 days, the range of triglyceride

in the kidney was only 2 to 4%. Whether the differences are of any biological significance is questionable. In any event, triglyceride was the one substance measured which most contributed to the increased kidney weight of the S 5B/Pl rats fed the high fat diet. Also, S 5B/Pl rats had equally as great a percentage of kidney triglyceride as did the Osborne-Mendel rats (Table 9).

There was no main effect of supplementation on kidney triglyceride (Table 5 and Figure 10). In regard to litter size, 24 day old rats of both strains combined that were raised six per litter had a mean kidney triglyceride value of 19.1 mg., or 3.12%. Rats of both strains combined that were raised three per litter had 19 mg. kidney triglyceride, or 3.08%. While litter size had exerted no effect at 24 days, there was a slight effect at 105 days. Rats of both strains raised six per litter had a mean triglyceride content of 64.6 mg. (3.06%), and those raised in litters of three, 56.2 mg. (2.57%). There was no significant five-way interaction of variables, nor were there any significant four-way interactions.



*Design followed for Osborne-Mendel and S5B/Pl male rats at both 24 and 105 days of age.

TABLE 1

COMPOSITION OF DIETS

Ingredients	g./100g. Diet LF	HF
Protein (casein)	22.0	33.00
Salt mix (Rogers and Harper)	4.0	6.00
Non-nutritive bulk (cellulose)	2.0	3.00
Vitamin mix	1.0	1.50
DL-methionine	0.1	0.15
Liver mix	1.5	2.20
Carbohydrate (cerelose)	66.4	10.40
Fat (Crisco)		43.75
(Corn Oil)	3.0	
Kcal/100g. ration	381.0	567.00
Kcal/g. protein	17.3	17.20

TABLE 2

TABLE 3

Live body weight, lean body mass, kidney (2) weight, kidney (g)/100g. body weight, kidney (g)/100g. lean body mass for 24 day old S 5B/Pl and Osborne-Mendel rats exposed to various treatments

Strain and Treatment	Rat, live wt. (g)	LBM wt. (g)	Kidney (2) wt.		
			Fresh wt. (g)	g/100g. BW	g/100g LBM
S, LF, NS, 6/L	47±2	37±1	0.48±0.04	1.02±0.04	1.31±0.05
S, LF, NS, 3/L	49±3	36±2	0.49±0.03	0.99±0.01	1.35±0.01
S, LF, Su, 6/L	47±3	36±3	0.48±0.03	1.04±0.07	1.34±0.14
S, LF, Su, 3/L	51±3	34±5	0.53±0.04	1.04±0.07	1.61±0.32
S, HF, NS, 6/L	50±3	38±3	0.54±0.02	1.07±0.05	1.43±0.10
S, HF, NS, 3/L	55±4	39±2	0.58±0.04	1.06±0.12	1.50±0.14
S, HF, Su, 6/L	49±2	37±1	0.54±0.03	1.10±0.04	1.47±0.04
S, HF, Su, 3/L	55±4	38±2	0.57±0.05	1.03±0.06	1.50±0.07
OM, LF, NS, 6/L	66±5	47±4	0.63±0.06	0.96±0.08	1.36±0.13
OM, LF, NS, 3/L	75±6	51±5	0.73±0.08	0.97±0.06	1.42±0.14
OM, LF, Su, 6/L	72±5	52±3	0.65±0.04	0.91±0.08	1.27±0.11
OM, LF, Su, 3/L	72±6	51±4	0.67±0.05	0.92±0.04	1.31±0.06
OM, HF, NS, 6/L	77±4	50±2	0.76±0.08	0.99±0.14	1.54±0.20
OM, HF, NS, 3/L	83±7	51±4	0.78±0.05	0.94±0.04	1.52±0.06
OM, HF, Su, 6/L	90±4	49±18	0.85±0.06	0.95±0.04	2.22±1.60
OM, HF, Su, 3/L	85±8	51±5	0.73±0.11	0.85±0.06	1.42±0.11

TABLE 4

Live body weight, lean body mass, kidney (2) weight, kidney (g)/100g. body weight, kidney (g)/100g. lean body mass for 105 day old S 5B/P1 and Osborne-Mendel rats exposed to various treatments

Strain and Treatment	Rat, live wt. (g)	LBM wt. (g)	Kidney (2) wt.		
			Fresh wt. (g)	g/100g. BW	g/100g.LBM
S, LF, NS, 6/L	279±7	215±8	1.99±0.10	0.71±0.02	0.92±0.04
S, LF, NS, 3/L	280±21	215±10	1.90±0.25	0.68±0.04	0.88±0.08
S, LF, Su, 6/L	259±17	218±48	1.74±0.19	0.67±0.03	0.81±0.13
S, LF, Su, 3/L	298±26	221±15	1.90±0.16	0.64±0.02	0.86±0.03
S, HF, NS, 6/L	282±18	222±14	2.19±0.10	0.78±0.02	0.99±0.03
S, HF, NS, 3/L	277±26	215±16	2.12±0.21	0.77±0.01	0.96±0.03
S, HF, Su, 6/L	329±52	236±30	2.38±0.40	0.72±0.03	1.01±0.05
S, HF, Su, 3/L	298±30	237±46	2.20±0.28	0.74±0.07	0.95±0.19
OM, LF, NS, 6/L	470±30	329±29	2.68±0.20	0.57±0.07	0.82±0.12
OM, LF, NS, 3/L	433±32	321±27	2.70±0.14	0.63±0.06	0.84±0.09
OM, LF, Su, 6/L	481±34	335±20	2.56±0.23	0.53±0.04	0.76±0.06
OM, LF, Su, 3/L	458±41	330±12	2.51±0.31	0.55±0.05	0.76±0.08
OM, HF, NS, 6/L	571±60	344±39	2.75±0.31	0.49±0.04	0.81±0.11
OM, HF, NS, 3/L	589±27	340±24	3.51±0.37	0.60±0.04	1.03±0.08
OM, HF, Su, 6/L	634±76	329±30	3.41±0.39	0.54±0.08	1.04±0.08
OM, HF, Su, 3/L	563±38	346±20	3.09±0.41	0.55±0.06	0.89±0.15

TABLE 5

RESULTS OF THE MULTIVARIATE ANALYSIS OF AGE, STRAIN, DIET, SUPPLEMENTATION AND LITTER SIZE ON BODY WEIGHT, KIDNEY (2) WEIGHT, AND KIDNEY DNA, PROTEIN AND TRIGLYCERIDE

Source of variation	df	Mean sq. of hypothesis	F value	P-less than
Body weight				
Age	1	4465073.6465	6245.4069	0.0001*
Strain	1	586041.7649	819.7108	0.0001*
Diet	1	42524.8615	59.4806	0.0001*
Supplementation	1	1387.6920	1.9410	0.1662
Litter size	1	9877.5679	13.8160	0.0004*
Interactions:				
2 way	10	50421.7199	70.5261	0.0001*
3 way	10	3557.2903	4.9757	0.0001*
4 way	5	1381.4075	1.9322	0.0939
5 way	1	229.9793	0.3217	0.5717
Kidney weight				
Age	1	130.5062	3510.4294	0.0001*
Strain	1	8.7419	235.1439	0.0001*
Diet	1	2.0462	55.0409	0.0001*
Supplementation	1	0.0058	0.1565	0.6931
Litter size	1	0.0437	1.1752	0.2805
Interactions:				
2 way	10	0.5856	15.7524	0.0001*
3 way	10	0.1136	3.0549	0.0018*
4 way	5	0.1213	3.2641	0.0085*
5 way	1	0.0613	1.6476	0.2018
DNA				
Age	1	189.8173	970.9182	0.0001*
Strain	1	28.3639	145.0817	0.0001*
Diet	1	6.0126	30.7543	0.0001*
Supplementation	1	0.1413	0.7225	0.3970
Litter size	1	0.0060	0.0304	0.8618
Interactions:				
2 way	10	2.0558	10.5152	0.0001*
3 way	10	0.4954	2.5339	0.0083*
4 way	5	0.4832	2.4718	0.0361
5 way	1	0.2564	1.3114	0.2545

TABLE 5--Continued

Source of variation	df	Mean sq. of hypothesis	F value	P-less than
Protein				
Age	1	1400176.0018	1568.1193	0.0001*
Strain	1	116127.7961	130.0567	0.0001*
Diet	1	16648.0414	18.6449	0.0001*
Supplementation	1	6520.0996	7.3021	0.0079*
Litter size	1	1623.8295	1.8186	0.1800
Interactions:				
2 way	10	8069.4217	9.0373	0.0001*
3 way	10	1461.4565	1.6368	0.1041
4 way	5	1304.4736	1.4609	0.2077
5 way	1	361.6080	0.4050	0.5258
Triglyceride				
Age	1	109680.2168	642.5258	0.0001*
Strain	1	4296.5223	25.1698	0.0001*
Diet	1	2595.2805	15.2036	0.0002*
Supplementation	1	162.2318	0.9504	0.3316
Litter size	1	1401.1060	8.2079	0.0050*
Interactions:				
2 way	10	646.2842	3.7860	0.0002*
3 way	10	231.2761	1.3549	0.2096
4 way	5	208.6060	1.2221	0.3030
5 way	1	445.6258	2.6106	0.1088

TABLE 6

TOTAL KIDNEY (2) DNA, PROTEIN AND TRIGLYCERIDE OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS EXPOSED TO VARIOUS TREATMENTS

Strain and Treatment	Total Kidney			Total Kidney		
	DNA (mg)	Protein (mg)	Triglyceride (mg)	DNA (mg)	Protein (mg)	Triglyceride (mg)
	24 days			105 days		
S, LF, NS, 6/L	1.85±0.22	53±9	16.0±4.8	4.01±0.48	235±40	65.4±12.9
S, LF, NS, 3/L	2.10±0.09	54±13	13.3±2.7	4.02±0.20	224±35	46.7±11.6
S, LF, Su, 6/L	2.09±0.15	53±5	14.9±3.3	3.75±0.23	204±22	55.4± 8.4
S, LF, Su, 3/L	2.30±0.14	59±7	16.4±2.7	4.22±0.53	223±48	50.8±25.9
S, HF, NS, 6/L	2.23±0.07	51±4	21.2±4.7	4.21±0.18	308±17	56.2± 4.1
S, HF, NS, 3/L	2.45±0.10	73±13	20.6±5.1	3.92±0.39	261±21	64.6± 7.5
S, HF, Su, 6/L	2.18±0.16	59±2	21.7±1.7	4.31±0.45	223±24	81.8±30.1
S, HF, Su, 3/L	2.20±0.24	53±7	19.5±4.2	4.00±0.30	237±57	77.5±12.4
OM, LF, NS, 6/L	2.36±0.15	79±4	14.6±7.1	5.42±0.33	352±56	89.5±24.2
OM, LF, NS, 3/L	2.65±0.22	84±22	22.2±1.0	5.31±0.34	347±37	70.0±14.5
OM, LF, Su, 6/L	1.99±0.47	58±9	14.8±1.7	5.05±0.60	309±33	93.7±13.0
OM, LF, Su, 3/L	2.28±0.36	73±16	16.2±5.1	4.91±0.66	296±28	60.3± 9.5
OM, HF, NS, 6/L	2.65±0.26	81±14	22.9±13.4	5.79±0.57	365±59	89.7± 9.2
OM, HF, NS, 3/L	2.74±0.18	90±9	25.1±6.0	7.25±2.05	389±54	77.0±10.4
OM, HF, Su, 6/L	2.89±0.21	94±8	26.3±2.6	6.95±1.22	418±93	87.8±29.0
OM, HF, Su, 3/L	2.72±0.39	85±12	22.7±7.7	6.27±0.66	395±93	95.5±25.6

TABLE 7

PROTEIN
g/100g. Kidney

24 Day Old Rats

	Osborne-Mendel				S 5B/P1			
	LF		HF		LF		HF	
	NS	Su	NS	Su	NS	Su	NS	Su
6/L	12.62	8.92	10.67	11.10	11.14	11.04	11.33	10.82
3/L	11.41	10.78	11.60	11.74	11.06	11.00	12.56	9.47

105 Day Old Rats

	Osborne-Mendel				S 5B/P1			
	LF		HF		LF		HF	
	NS	Su	NS	Su	NS	Su	NS	Su
6/L	13.10	12.13	13.25	12.16	11.82	11.84	14.05	9.48
3/L	12.88	11.86	11.06	12.70	11.89	11.63	12.30	10.74

TABLE 8

PROTEIN/DNA
(mg./mg.)

24 Day Old Rats

	Osborne-Mendel				S 5B/P1			
	LF		HF		LF		HF	
	NS	Su	NS	Su	NS	Su	NS	Su
6/L	33	29	31	33	29	26	27	27
3/L	32	32	33	31	26	25	30	24

105 Day Old Rats

	Osborne-Mendel				S 5B/P1			
	LF		HF		LF		HF	
	NS	Su	NS	Su	NS	Su	NS	Su
6/L	65	61	63	60	59	54	73	52
3/L	65	60	54	63	56	53	67	59

TABLE 9

TRIGLYCERIDE
g/100g. Kidney

24 Day Old Rats

	Osborne-Mendel				S 5B/P1			
	LF		HF		LF		HF	
	NS	Su	NS	Su	NS	Su	NS	Su
6/L	2.27	2.27	2.91	3.10	3.36	3.07	3.95	3.99
3/L	3.11	2.40	3.24	3.08	2.73	3.11	3.56	3.43

105 Day Old Rats

	Osborne-Mendel				S 5B/P1			
	LF		HF		LF		HF	
	NS	Su	NS	Su	NS	Su	NS	Su
6/L	3.31	3.67	3.29	2.59	3.28	3.20	2.57	3.36
3/L	2.61	2.40	2.23	3.10	2.47	2.61	3.06	3.59

FIGURE 1

MEAN BODY WEIGHTS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS FED EITHER A HIGH FAT OR A LOW FAT DIET

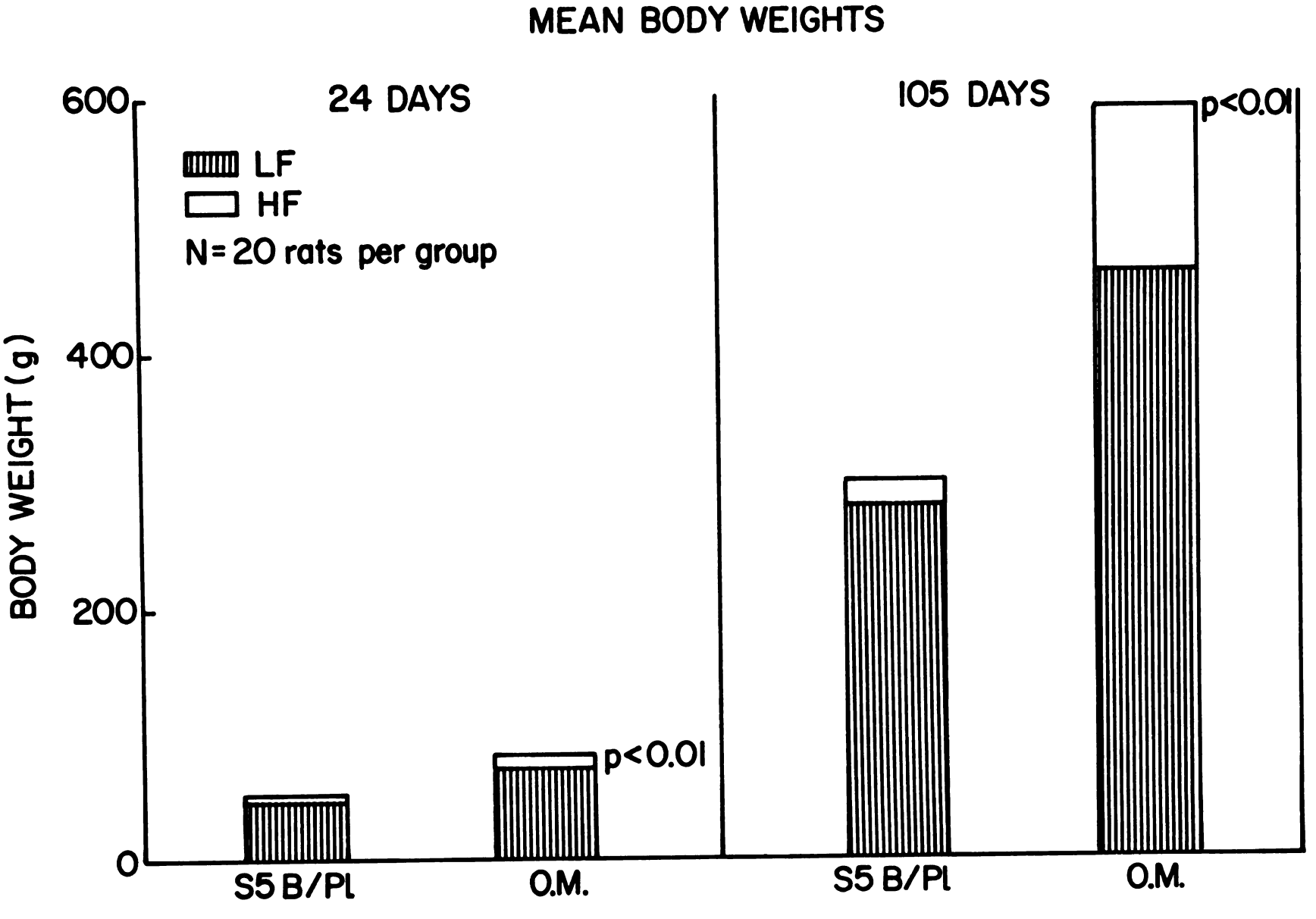

MEAN BODY WEIGHTS
24 DAYS
105 DAYS
LF
HF
N=20 rats per group
BODY WEIGHT (g)
600
400
200
0
p<0.01
p<0.01
S5 B/Pl
O.M.
S5 B/Pl
O.M.

FIGURE 2

TOTAL BODY WEIGHTS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS EXPOSED TO VARIOUS TREATMENTS

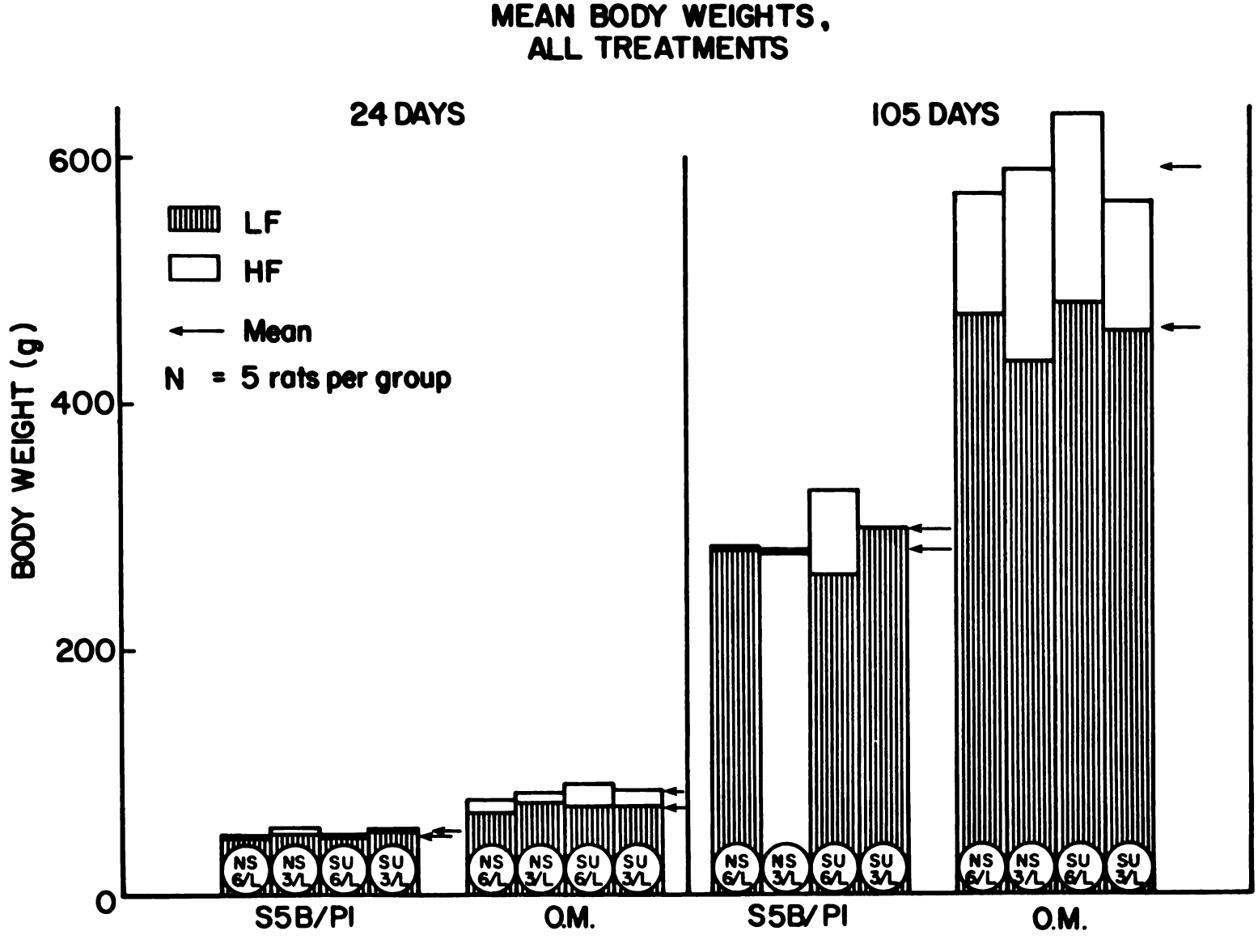

MEAN BODY WEIGHTS,
ALL TREATMENTS
24 DAYS
105 DAYS
BODY WEIGHT (g)
600
400
200
0
LF
HF
Mean
N = 5 rats per group
NS 6/L
NS 3/L
SU 6/L
SU 3/L
S5B/PI
O.M.
S5B/PI
O.M.

FIGURE 3

MEAN KIDNEY (2) WEIGHTS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS FED EITHER A HIGH FAT OR A LOW FAT DIET

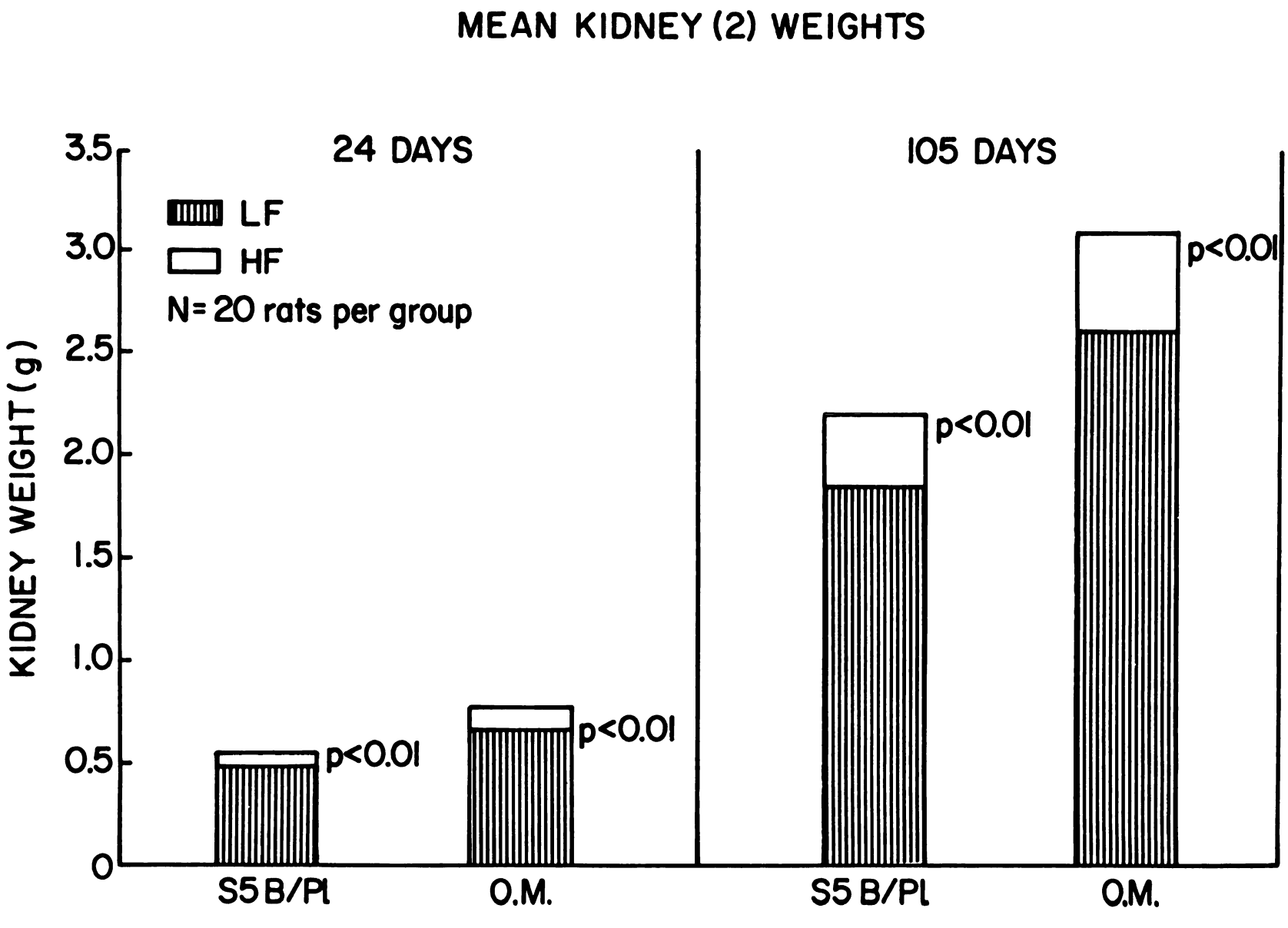

MEAN KIDNEY (2) WEIGHTS
24 DAYS
105 DAYS
LF
HF
N= 20 rats per group
KIDNEY WEIGHT (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
p<0.01
p<0.01
p<0.01
p<0.01
S5 B/Pl
O.M.
S5 B/Pl
O.M.

FIGURE 4

TOTAL KIDNEY WEIGHTS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS EXPOSED TO VARIOUS TREATMENTS

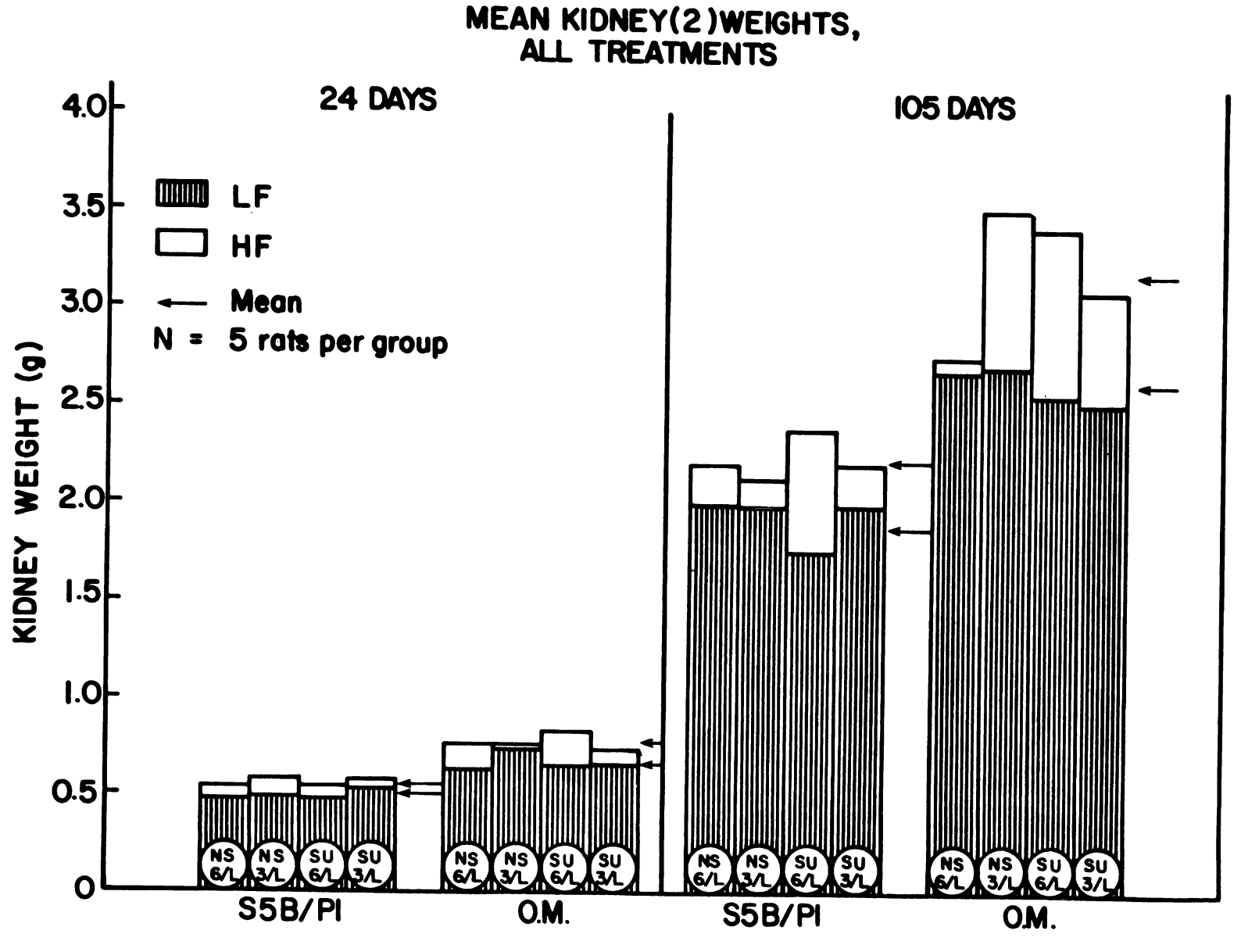

MEAN KIDNEY(2)WEIGHTS,
ALL TREATMENTS
24 DAYS
105 DAYS
LF
HF
Mean
N = 5 rats per group
KIDNEY WEIGHT (g)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
NS 6/L
NS 3/L
SU 6/L
SU 3/L
S5B/PI
O.M.

FIGURE 5

MEAN KIDNEY (2) DNA OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS FED EITHER A HIGH FAT OR A LOW FAT DIET

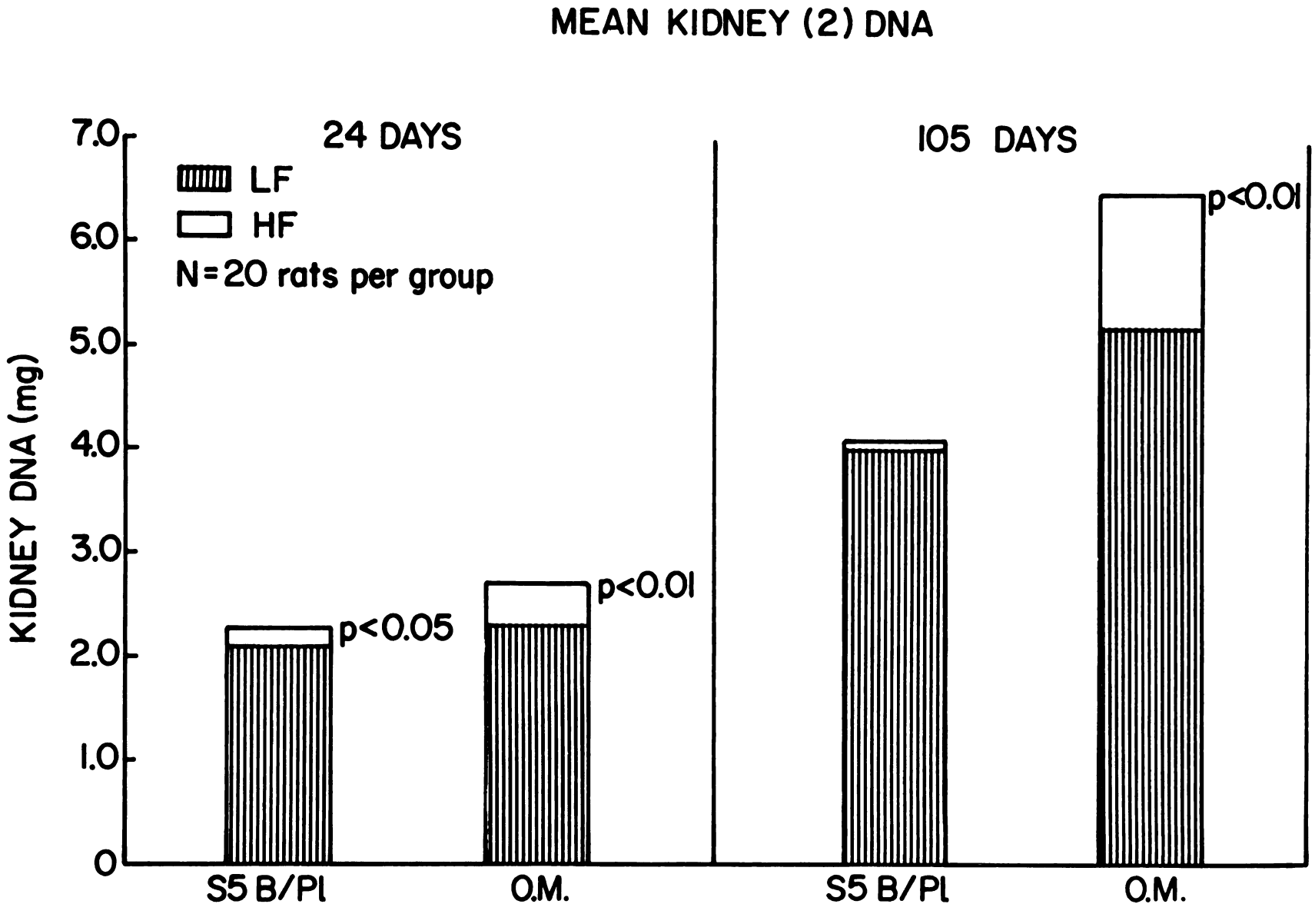

MEAN KIDNEY (2) DNA
24 DAYS
105 DAYS
LF
HF
N=20 rats per group
KIDNEY DNA (mg)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
p<0.05
p<0.01
p<0.01
S5 B/Pl
O.M.
S5 B/Pl
O.M.

FIGURE 6

TOTAL DNA IN KIDNEYS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS EXPOSED TO VARIOUS TREATMENTS

MEAN KIDNEY (2) DNA,
ALL TREATMENTS

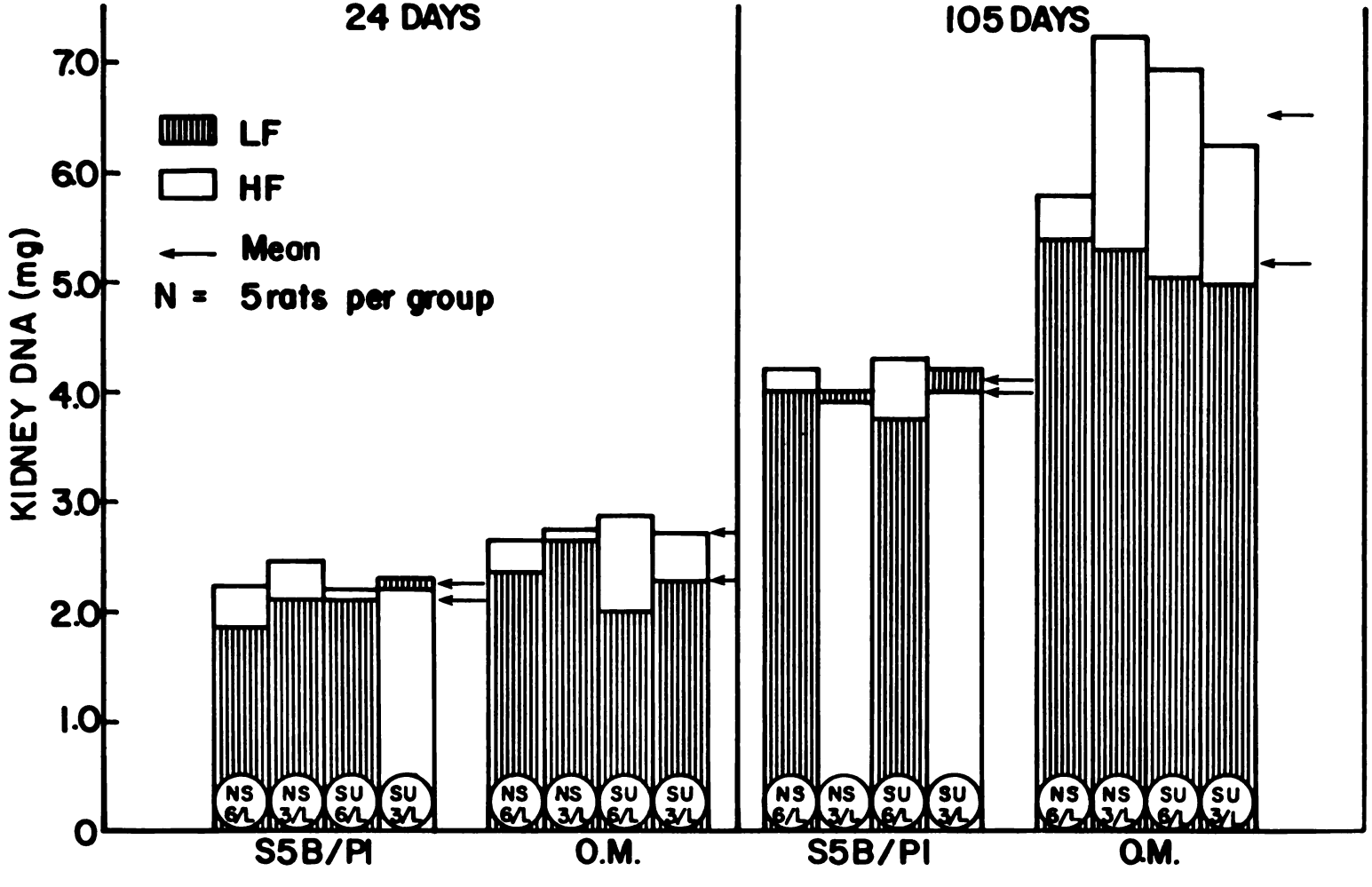


FIGURE 7

MEAN KIDNEY (2) PROTEIN OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS FED EITHER A HIGH FAT OR A LOW FAT DIET

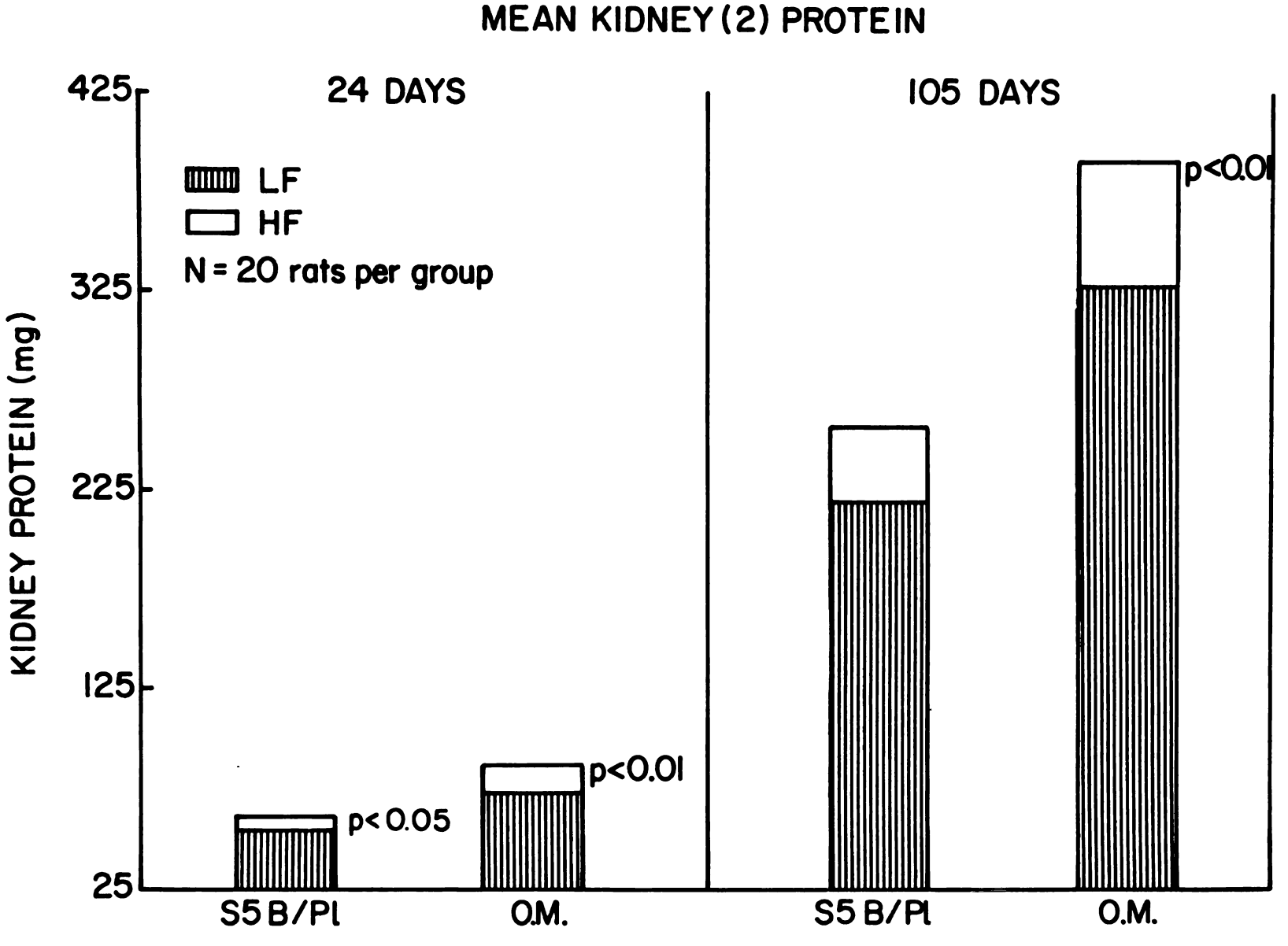

MEAN KIDNEY (2) PROTEIN
24 DAYS
105 DAYS
LF
HF
N = 20 rats per group
KIDNEY PROTEIN (mg)
425
325
225
125
25
p< 0.05
p<0.01
p<0.01
S5 B/Pl
O.M.
S5 B/Pl
O.M.

FIGURE 8

TOTAL PROTEIN IN KIDNEYS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS EXPOSED TO VARIOUS TREATMENTS

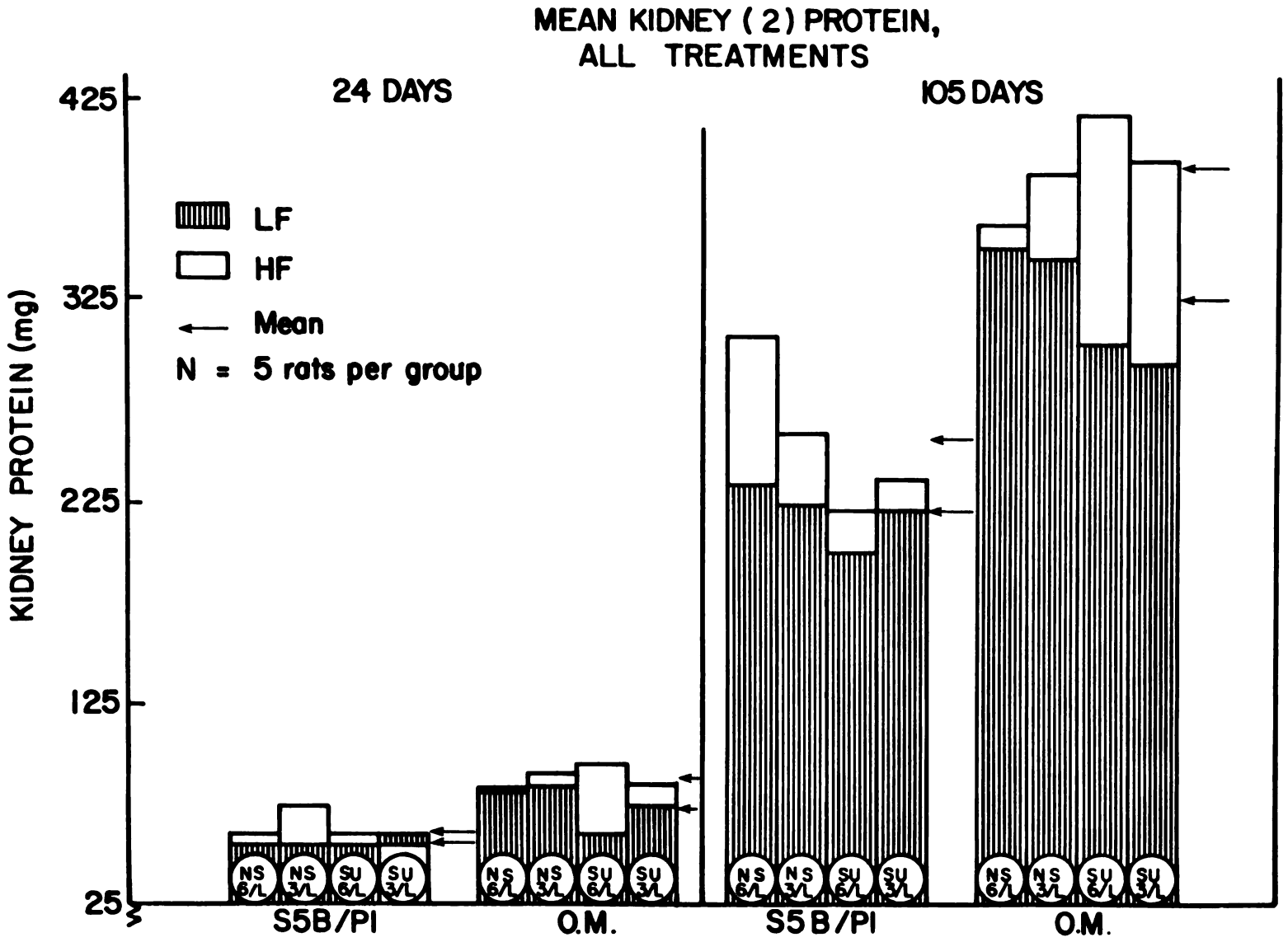

MEAN KIDNEY (2) PROTEIN,
ALL TREATMENTS
24 DAYS
105 DAYS
KIDNEY PROTEIN (mg)
425
325
225
125
25
LF
HF
Mean
N = 5 rats per group
NS 6/1
NS 3/1
SU 6/1
SU 3/1
S5B/PI
O.M.
S5B/PI
O.M.

FIGURE 9

MEAN KIDNEY (2) TRIGLYCERIDE OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS FED EITHER A HIGH FAT OR A LOW FAT DIET

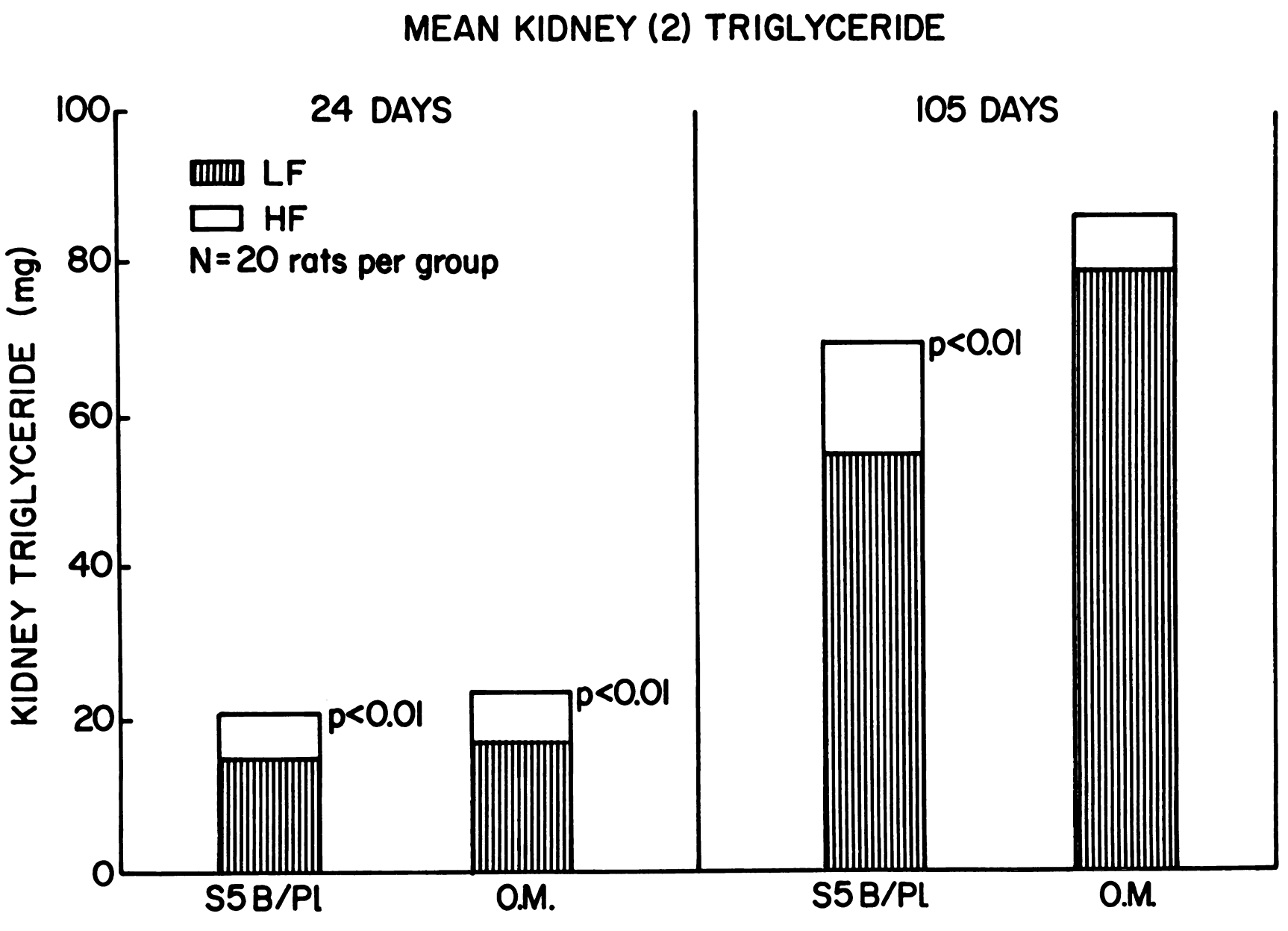
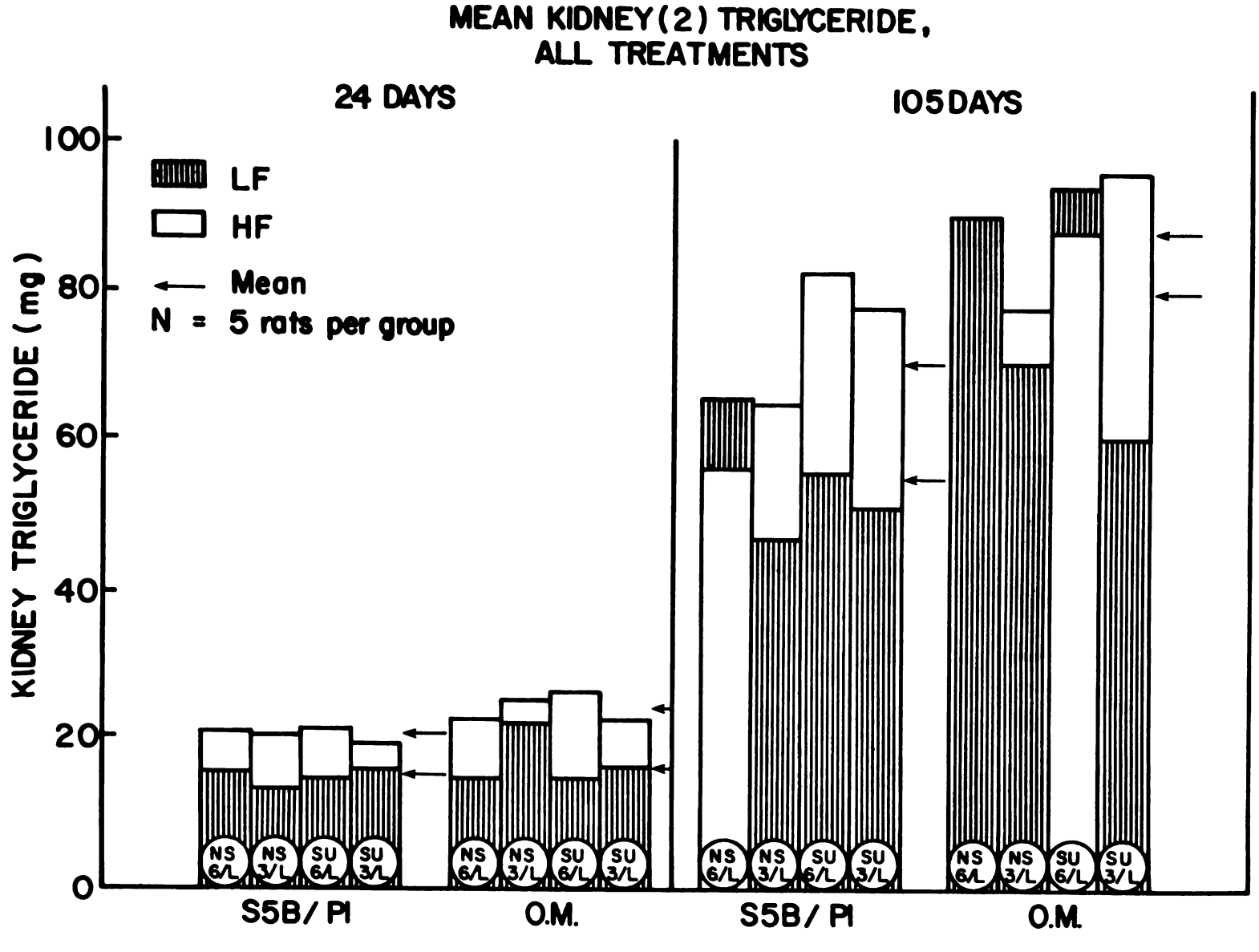

MEAN KIDNEY (2) TRIGLYCERIDE
24 DAYS
105 DAYS
LF
HF
N=20 rats per group
KIDNEY TRIGLYCERIDE (mg)
100
80
60
40
20
0
p<0.01
p<0.01
p<0.01
S5 B/Pl
O.M.
S5 B/Pl
O.M.

FIGURE 10

TRIGLYCERIDE CONTENT OF KIDNEYS OF 24 AND 105 DAY OLD S 5B/P1 AND OSBORNE-MENDEL RATS EXPOSED TO VARIOUS TREATMENTS


MEAN KIDNEY(2) TRIGLYCERIDE,
ALL TREATMENTS
24 DAYS
105 DAYS
KIDNEY TRIGLYCERIDE (mg)
100
80
60
40
20
0
LF
HF
Mean
N = 5 rats per group
NS 6/L
NS 3/L
SU 6/L
SU 3/L
S5B/ Pl
O.M.

CONCLUSIONS

Age

Although the ages of 24 and 105 days are not ideal for the study of renal growth exclusively, they are acceptable in light of the fact that the original research was designed with several purposes in mind. The overall growth of whole body and individual organs and tissues was to be assessed in relation to the influence of early overfeeding practices. One must remember when reviewing these data, that nephrogenesis in the rat is complete by three or four weeks of age (Kittelson, 1917; Arataki, 1926; Boss et al., 1963; Bengele and Solomon, 1974). Because cell division is complete on or around 44 days (Winick and Noble, 1965) it must be realized that any increase in DNA occurs before then. In reference to protein, its accumulation begins to plateau at about 45 or 50 days of age (Priestley and Malt, 1968).

The variable, Age, led to an increase in all indices measured. All animals in both strains had kidneys at 105 days which weighed four times as much as they had at 24 days. The increase in weight was reflected by a doubling in the number of cells, and in the protein/DNA ratio. These findings are similar to those of Winick and Noble (1965, 1967).

Strain

The S 5B/Pl and Osborne-Mendel rats responded differently to experimentally induced overnutrition begun in the suckling period.

By weaning, Osborne-Mendel pups that had been nursed by dams fed a high fat diet weighed 13 g. more than those nursed by dams fed a low fat diet. The respective difference in the S 5B/Pl strain was 3 g. The strain difference was even more apparent by 105 days when the difference between the experimental and control Osborne-Mendel rats was 128 g. and between the S 5B/Pl rats, 18 g. Clearly the Osborne-Mendel rat is a good model for studying the ramifications of overnutrition. In this species of animal there appears to be a genetic difference in the propensity toward obesity, as evidenced by the response of the S 5B/Pl rat. Providing this strain of rat with a more calorically dense milk during the suckling period and a post-weaning calorically dense diet, to say nothing of force-feeding and reducing litter size, had no effect on the rate of growth. Schemmel et al. (1970) did, in fact, find that body weight gain in seven strains of rats fed a high fat (60% w/w) diet was influenced by genetics. The S 5B/Pl rat showed minimal response. In a later study, Schemmel et al. (1972) showed that the S 5B/Pl rat eats less food than the Osborne-Mendel, and converts food calories to body energy less effectively. Fenton and Carr (1951) also found in mice that the effect of the fat content of the ration on weight gain was dependent upon the strain of animal employed.

Diet

The overfeeding technique which exerted the greatest influence on growth was feeding a high fat diet. Osborne-Mendel rats maintained on a high fat diet had significantly greater body and kidney weights at both ages than did their controls. The increased kidney weight at 24 days was primarily the result of the organ containing a greater number

of cells, although triglyceride was also increased. The greater kidney weight at 105 days was also due to the acquisition of more cells, the organs of the experimental group containing 27% more DNA than those of the controls. There was no difference at either age in the protein/DNA ratio between Osborne-Mendel rats fed high fat or low fat diets, indicating that the cells were of the same size.

It may be concluded that feeding the Osborne-Mendel rat a high fat diet from birth caused accelerated growth of the kidney as well as the whole body. The more pronounced growth of the kidney was a function of cell division. These findings are in agreement with those of Winick and Noble (1967), who concluded that increasing the number of calories available to the young rat accelerates growth by increasing the rate of cell division.

Supplemental Feeding and Litter Size Reduction

Supplemental force-feeding and litter size reduction have each been effective in accelerating growth, when used independently. Whether either of these techniques was influential to the outcome of this study is questionable (see also, Harris et al., 1977). The over-riding effect of feeding a high fat diet may have masked the contribution of either technique.

The technical details of force feeding, in this experiment, were implemented and supervised by one who had perfected the method (Czajka-Narins, 1974), ruling out the possibility of faulty delivery of the supplement. Giving more than two supplemental feedings a day, or using a random schedule may have been helpful. The latter adjustment would rule out the possibility that the pups anticipated the feedings and

decreased their food intake beforehand (Wurtman and Miller, 1976b). Supplemental force-feeding may have also indirectly affected the amount of milk available from the dams by causing the satiated pups to suck less and thus diminish the stimulus for increased milk production.

It has not been clearly confirmed that growth in the rat is inversely related to litter size due to the availability of milk. To the contrary, Winick and Noble (1967) found no difference in growth parameters of Sprague Dawley rats raised three or six per litter. Clearly growth rate may be influenced by using extremes in litter size (Widdowson and McCance, 1960). Also, the work of Winick and Noble (1967) shows that there are differences in the growth of rats raised twelve and six per litter. Perhaps raising rats in litters of three diminishes the suckling stimulus needed for milk production and makes less milk available to them than to those raised in litters of six.

APPENDIX

APPENDIX

SOLUTIONS USED IN THE ANALYSIS OF KIDNEY DNA AND PROTEIN

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SOLUTIONS USED IN THE ANALYSIS OF KIDNEY DNA AND PROTEIN

Solutions Used in the Analysis of Kidney DNA

1. 20% TCA: 200 g. of trichloroacetic acid[1] were weighed on a Mettler top loading balance,[2] and transferred to a 1,000 ml. volumetric flask. Enough distilled deionized[3] water was added for thorough mixing, and then for making the solution to volume. The solution was mixed by inversion and stored in the refrigerator in 1 liter glass bottles.
2. 10% TCA: 100 g. of trichloroacetic acid were weighed on a Mettler top loading balance, and the solution was made to volume and stored as above.
3. 5% TCA: 50 g. of trichloroacetic acid were weighed and made to volume as above. The solution was stored at room temperature in 1 liter glass bottles.
4. NaOH, 1 pellet/liter: One pellet of sodium hydroxide[4] was placed in a 1,000 ml. volumetric flask. A small amount of

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[1]Analytic reagent, Mallincrodt Chemical Works, St. Louis, MO.

[2]Mettler P 1200, Mettler Instrument Corporation, Hightstown, N.J.

[3]Illco-Way Ion XChanger, Research Model I, Illinois Water Treatment Co., 840 Cedar Street, Rockford, Ill.

[4]USP, J. T. Baker Chemical Co., Philipsburg, N.J.

distilled deionized water was added, and the flask shaken until the pellet dissolved. The solution was made to volume with distilled deionized water, mixed, and stored at room temperature in a 1 liter glass bottle.

5. Diphenylamine-acetaldehyde solution: 100 ml. of glacial acetic acid[5] was measured into a graduated cylinder. To this was added 1.5 g. of crystalline diphenylamine.[6] The cylinder was stoppered, and the contents shaken by hand until all of the diphenylamine was in solution. Then 1.5 ml. of concentrated H_2SO_4[7] was added by pipette. The contents were shaken, and 100 ml. was transferred into a 100 ml. volumetric flask. The solution was made fresh daily, and was kept out of the light until it was used. Just before using, 0.5 ml. of refrigerated acetaldehyde[8] solution (aqueous, 16 mg./ml.) was added to the volumetric flask containing the diphenylamine solution, and the contents were shaken.

6. DNA standard: 10 mg. of calf thymus DNA[9] was cut, weighed and transferred into a 100 ml. volumetric flask. 50 ml. of a "one pellet/liter" solution of NaOH[10] was added in order to dissolve

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[5]ACS reagent, A38-C, Fisher Scientific Co., Fair Lawn, N.J.

[6]No. 4938, Analytical reagent, Mallinckrodt Chemical Works, St. Louis, Mo.

[7]No. 3-9681, J. T. Baker Chemical Co., Phillipsburg, N.J.

[8]No. 2401, Mallinckrodt Chemical Works, St. Louis, Mo.

[9]Type 1, No. D 1501, Sigma Chemical Corporation, P.O. Box 4508, St. Louis, Mo.

[10]USP, J. T. Baker Chemical Co., Phillipsburg, N.J.

the DNA. After several hours and intermittent mixing with a stirring rod, the DNA was dissolved. The solution was then made to volume with 50 ml. of 10% TCA. The white precipitate which formed, disappeared upon gently heating the flask in an asbestos heating mantle. The final solution had a concentration of 100 ug./ml. It was stored in a plastic container at 0 degrees centigrade. The following protocol was followed in diluting the standard for preparation of the standard curve:

ug. DNA/ml.	ml. DNA Standard	ml. 5% TCA
0	0	2.0
10	0.2	1.8
20	0.4	1.6
30	0.6	1.4
70	1.4	0.6
100	2.0	0

Solutions Used in the Analysis of Kidney Protein

1. 10% TCA: The solution was prepared as under Solutions Used in the Analysis of Kidney DNA.
2. 1 N NaOH: 40 g. of sodium hydroxide were weighed on the Mettler top loading balance, and transferred to a 1,000 ml. volumetric flask. Enough distilled deionized water was added for thorough mixing, and then for making the solution to volume. The solution was mixed by inversion and stored at room temperature in 1 liter plastic bottles.
3. 0.5% $CuSO_4$: 0.5 g. of $CuSO_4 \cdot 5H_2O$[11] was weighed on the Mettler

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[11]Analytical Reagent, No. 4844, Mallinckrodt Chemical Works, St. Louis, Mo.

analytical balance[12] and transferred to a 100 ml. volumetric flask. Enough distilled deionized water was added for thorough mixing, and then for making the solution to volume. The solution was mixed by inversion, and stored at room temperature in 100 ml. plastic bottles.

4. 2% Na_2CO_3--0.02% $Na_2C_4H_4O_6 \cdot 2H_2O$: 20 g. of sodium carbonate[13] was weighed on the Mettler top loading balance, and transferred to a 1,000 ml. volumetric flask. 0.2 g. of sodium tartrate[14] was weighed on the Mettler analytical balance, and transferred to the same flask. Enough distilled deionized water was added to the flask for thorough mixing, and then for making the solution to volume. The solution was mixed by inversion, and stored at room temperature in 1 liter bottles.

5. Copper sulfate-sodium carbonate-sodium tartrate solution: Just before treating the samples and standards, 2 ml. of 0.5% $CuSO_4 \cdot H_2O$ stock solution was transferred by pipette to a 100 ml. volumetric flask. The solution was made to volume with the 2% sodium carbonate--0.02% sodium tartrate solution. The final solution was used for one day only.

6. Folin-Ciocalteau solution, 1N: Just prior to the treatment of the samples and standards, 10 ml. of 2N Folin-Ciocalteau reagent[15] was transferred by volumetric pipette into a 25 ml. beaker. Ten

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[12]Type H 15, Mettler Instrument Corporation, Hightstown, N.J.

[13]Anhydrous, No. 3602, J. T. Baker Chemical Co., Phillipsburg, N.J.

[14]Crystalline, J. T. Baker Chemical Co., Phillipsburg, N.J.

[15]Phenol Reagent Solution 2N (Folin-Ciocalteau), SO-P-24, Fisher Scientific Co., Fair Lawn, N.J.

mililiters of distilled deionized water was in like manner, transferred to the beaker. The addants were mixed well and added to the samples and standards at the appropriate time.

7. Protein standard stock solution: Two hundred and fifty miligrams of crystalline bovine albumin[16] was weighed and transferred to a 50 ml. volumetric flask. Approximately 20 ml. of distilled deionized water was added and the flask inverted until the albumin was dissolved. The solution was made nearly to volume with more distilled deionized water, and then the flask was stoppered and refrigerated overnight. By the next day the foam which had formed during dilution and mixing had disappeared, and the solution was made to volume with distilled deionized water. The standard was stored in a plastic bottle at 0 degrees centigrade, and was diluted 1:10 with 1N NaOH before use.

The following protocol was followed in diluting the standard for preparation of the standard curve:

ug. Protein/ml.	ml. Protein Standard	ml. Water
50	0.1	0.9
100	0.2	0.8
150	0.3	0.7
200	0.4	0.6
250	0.5	0.5
300	0.6	0.4

[16]No. 7285, Nutritional Biochemicals Corporation, Cleveland, Ohio.

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