EFFECT OF DIET AND CONCENTRATION OF NaCl IN DRINKING SOLUTIONS ON BLOOD PRESSURE, PULSE RATE SODIUM AND POTASSIUM CONTENT OF SELECTED TISSUES IN RATS

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ABSTRACT

EFFECT OF DIET AND CONCENTRATION OF NaCl IN DRINKING SOLUTIONS ON BLOOD PRESSURE, PULSE RATE SODIUM AND POTASSIUM CONTENT OF SELECTED TISSUES IN RATS

By

Christine Gustara Beebe

There is evidence that the type of dietary carbohydrate ingested by rats may alter blood pressure (Hall et al. 1966, Yudkin 1972). There is also evidence in a number of studies of a positive blood pressure elevating effect of sodium chloride in rats fed chow-type diets (Dahl 1961, 1967, 1972, Sapirstein et al. 1950, Lenel et al. 1948). The purpose of the present study was to examine the effect of various diets on blood pressure and pulse rate and to note any effect of a salt-diet interaction on these parameters. In addition examination of growth rate, specific tissue size and tissue electrolyte concentration was determined in an effort to explain any blood pressure changes observed.

Sixty-four male Osborne Mendel rats were divided into 4 groups of 16 animals each. Each group was fed 1 of 4 diets consisting of primarily grain, fat (51.7%), sucrose (67%) or cornstarch (67%). The diets were allowed ad libitum beginning at 21 days of age for a period of 10 weeks.

Each group of rats was further divided into 2 groups of 8 rats each and offered a drinking solution of either 2% NaCl and water or distilled deionized water. Mean systolic blood pressure and pulse rates were recorded weekly for the 10 week feeding period. At the end of the feeding period the animals were sacrificed for body composition analysis and determination of tissue electrolyte concentration.

In a second experiment, 24 rats were divided into 4 groups of 6 rats each and fed 1 of the 4 diets used in the previous experiment. The rats were offered a 1% NaCl and water drinking solution for 9 weeks post-weaning followed by a 1.5% NaCl solution for 9 additional weeks. Mean systolic blood pressure and pulse rates were recorded weekly until the rats were sacrificed after 18 weeks of feeding.

Total diet consumption expressed as kcal. was nearly equivalent in rats given distilled deionized water regardless of the type of diet fed. Total kcal. consumption was less in rats allowed the 2% NaCl solution irrespective of the type of diet fed. This decrease in food consumption ranged from 20% in grain-fed rats to 58% in rats fed the high sucrose diet.

Total fluid consumption was similar in rats given distilled deionized water regardless of diet, in contrast however, consumption increased 3-fold in rats given the 2% NaCl solution. Rats given the 1-1.5% NaCl solution consumed approximately the same quantity of fluid regardless of the type of diet fed. When total sodium intake was

calculated relative to body weight, sodium intake of rats given the distilled deionized water was similar and ranged from 1.7 to 2.1 gm/100 gm body weight regardless of the type of diet fed. In contrast, rats given the 2% NaCl drinking solution exhibited greater variability in sodium intake ranging from 26.5 gm/100 gm body weight in rats fed the high fat diet to 53.4 gm/100 gm body weight in rats fed the high sucrose diet. Rats fed either grain or the high cornstarch diet consumed 32.9 and 29.9 gm/100 gm body weight, respectively.

In general, growth rates for all rats reflected the total amount of energy consumed. Rats given the 2% NaCl solution had very low body weights when compared to rats given distilled deionized water. In contrast, rats allowed the 1-1.5% NaCl drinking solution did not appear significantly stunted in rate of growth.

Body composition revealed that rats given the 2% NaCl solution had very little body fat (6-9%) regardless of the diet fed. Rats given the distilled deionized water contained larger and more variable amounts of fat; i.e., 9%, 24%, 20% and 15% for rats fed grain, high fat, high sucrose and high cornstarch diets, respectively. Total body water remained similar in rats given either distilled deionized water or the 2% NaCl drinking solution irrespective of diet.

Final mean blood pressures were elevated to hypertensive levels in rats given the 2% NaCl solution and fed the high fat, high sucrose or high cornstarch diets (154,

178 and 150 mmHg respectively). Blood pressure was elevated in rats fed the grain diet (127 mmHg), however, not to the hypertensive level. Rats given the distilled deionized water remained normotensive. However, rats fed the high sucrose diet and given distilled deionized water had significantly higher blood pressures than rats fed the grain diet ($P < .01$). A similar trend was observed in rats offered the 1-1.5% NaCl drinking solution, i.e., blood pressures remained within the normal range for rats fed grain, high fat and high cornstarch diets but were elevated to the range for hypertension in rats fed the high sucrose diet.

Pulse rates were variable in rats given the 2% NaCl solution. Regardless of the type of drinking solution consumed, the pattern of pulse rate over time was similar in all groups of rats, i.e., rats fed the high sucrose diet maintained the highest pulse rate.

Relative to body weight, the heart, right kidney and right adrenal gland were hypertrophied in those rats offered the 2% NaCl drinking solution. The greatest amount of hypertrophy of the heart and kidney was observed in rats fed the high sucrose diet whereas adrenal hypertrophy was greatest in rats fed the high fat diet.

Sodium and potassium concentrations in the serum and heart remained constant in all animals regardless of the type of diet or drinking solution. Concentration of sodium in the kidney increased with increase in sodium intake and therefore was largest in rats offered the 2% NaCl drinking

solution. Concentration of potassium remained unchanged in the kidney. The bone of the rat appears to be a labile source of sodium since sodium concentration in the femur increased markedly above normal normal values of 139-156 meq per gram of dry fat-free bone (57-236%) as sodium intake relative to body weight increased. For the most part, concentration of potassium in the femur decreased as sodium concentration increased.

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To my husband

Dick

and my son

Nathan

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REVIEW OF LITERATURE

Hypertension--Implications to Health

Cardiovascular Risk Factor

Elevated blood pressure is one of the major risk factors contributing to cardiovascular disease. Hypertension, as it is commonly called, is a result of various known or unknown factors which increase cardiac output or more often increase periferal resistance in the circulation (Ganong 1971). Regardless of how hypertension is initiated the heart must work harder for each volume of blood pumped through the system. Therefore, any disease or complication in the heart has more severe consequences in hypertensive individuals.

Hypertensives are also predisposed to thrombosis of cerebral vessels and cerebral hemorrhage (Ganong 1971, McMahon 1973). In addition renovascular disease (Ledingham 1971) and premature onset of atherosclerosis (Brest and Moyer 1967) are greatly increased in hypertension.

Statistics have dramatically highlighted the earlier mortality and increased morbidity of individuals with elevated blood pressure as compared to individuals with normal blood pressure levels. Actuarial survival rates published by life insurance companies (Actuarial Society of America 1940 and 1941) indicate that at any age in the adult

the risk to life from the complications of high blood pressure increases steadily as pressure exceeds the mean of the population.

Defining Hypertension

The term hypertension has been difficult to define since a temporary rise in blood pressure can occur under a variety of situations such as physical and emotional stress. Customarily the term applies to the situation of an increase in mean arterial blood pressure in a patient at rest in both mind and body (Ledingham 1971). Herein lies a problem since it is difficult to determine when an individual is at rest in mind.

Hypertension has also been defined as a level of blood pressure in an individual which lies 2, 3 or more standard deviations above the mean blood pressure in the population from which the individual is drawn, allowing for age and sex (Ledingham 1971). This definition appears illogical since the population as a whole, due to heredity or environment, may have a higher blood pressure than other populations.

Various limits of normal blood pressure have been established by various individuals in numerous investigations. Dahl (1972) measured blood pressure of employees at Brookhaven National Laboratories and established 140 mmHg systolic and 90 mmHg diastolic pressure as the upper limits for normal blood pressure in the subjects. A study of hypertensives in the inner city of New Orleans (McMahon 1973) defined hypertension as a pressure greater than 150/90 mmHg.

A blood pressure of 160/95 mmHg has been used in some instances to indicate hypertension (Chiang et al. 1969).

Studies using rats in developing experimental hypertension have defined hypertension as a systolic pressure of 150 mmHg (Molteni and Brownie 1972), 140 mmHg (Dahl 1961, Goldman et al. 1972), or 136 mmHg (Sapirstien et al. 1950).

Obviously, the frequency of hypertension in a given population is dependent upon the level of blood pressure one chooses to define as the line between "normal" and "abnormal". This appears to vary between investigators. The fact remains however, that an increase in blood pressure above normal is recognized as detrimental to the health of the individual (McMahon et al. 1973, Ledingham 1971).

Classifying Hypertension

Hypertension can be classified into either primary (essential) or secondary hypertension. Secondary hypertension involves individuals with elevated blood pressure due to a variety of diseases such as renal failure, glomerulonephritis and adrenocortical diseases (Ledingham 1971, Ganong 1971). Quite often this type of hypertension can be alleviated if the affecting disease is cured. Hypertension due to any of a variety of diseases can enter an accelerated phase in which necrotic arteriolar lesions develop and eventually lead to a malignant form of hypertension.

Essential hypertension is the most common form of hypertensive disease and involves 90% of persons with the disease (Goldman et al. 1972). As the name implies, this

elevation in blood pressure is characterized by diffuse arteriolar constriction of unknown cause (Ganong 1971). In an effort to define the etiology of the disease several contributing factors have been implicated, of which heredity and environment are the two most often pursued.

The importance of heredity to the development of hypertension has been demonstrated in both human and animal studies. The incidence of hypertension in the American Negro for example, has been shown to be more frequent than in the American White for both sexes (McMahon et al. 1973). It was once thought that the American Negro acquired this propensity for hypertension upon entering western civilization. A study of healthy Bantu males in South Africa indicated the contrary however (Dahl 1972), when a high incidence of hypertension was found in this population group.

Several strains of laboratory rats have been selectively inbred to produce offspring with genetic spontaneous hypertension of greater than 150 mmHg systolic pressure (Smirk and Hall 1958, Okamoto and Aoki 1963, Dahl et al. 1967). Dahl et al. (1967), through selective inbreeding developed two strains of rats with opposite genetic propensities for hypertension when fed a high salt diet. The sensitive or (S) strain developed hypertension quite readily whereas the resistant or (R) strain did not. Data from parabiotic union of R and S rats allowed Dahl to postulate that the difference between the two strains was the result

of an undefined humoral mechanism. Further evidence to verify this postulation has not been produced.

Stress, physical work and diet have been implicated as the major environmental factors contributing to the development of essential hypertension (Ledingham 1971). Diet has been approached with the greatest degree of vigor and has involved such parameters as coffee drinking, obesity, trace elements, fat, protein and carbohydrate intake and salt ingestion.

Age and Hypertensive Disease

McMahon et al. (1973) studied 11,309 human subjects of differing age, race and sex and found that hypertension increased with age (arterial pressure greater than 150/90 mmHg). Of the subjects in the 50-59 age category, 57% of them were hypertensive. Of those in the 60 and above age category, 70% were hypertensive.

Russek et al. (1946) studied blood pressures of 5,331 men between the ages of 40 and 95 years. He observed that the frequency of systolic hypertension rose sharply with advancing years. Diastolic blood pressure however, showed little variation after the sixth decade.

Blood pressures of Wistar Albino and Gray rats were studied over time by Durant (1927). He found systolic blood pressure increased with age in the Albino rat but not in the gray strain. Accurate data for blood pressure changes in the rat over time has been reported by Heyman and Salehar (1949). They noted that systolic blood pressure increased

with age in the growing rat. Within the first 2 months up to a body weight of 150 grams the blood pressure increased rapidly. A slower but more progressive rise was noted until 4 months of age or 350 grams of body weight after which time blood pressure stabilized.

Obesity and Hypertension

Clinical studies have indicated a definate correlation between obesity and increased systolic blood pressure (Wood and Cash 1939). Kannel and his coworkers (1967) reported information obtained from the Framingham study concerning the development of hypertension. They noted that over a 12 year period, normotensive obese individuals developed hypertension more often than normotensive individuals near ideal body weight. The risk of hypertension was 8 times greater in those individuals 20% overweight as opposed to those 10% underweight.

A study by Hartman and Grist (1929) demonstrated a steplike rise in systolic blood pressure in subjects ranging from 25% underweight to 25% overweight. The greatest difference in blood pressure occurred between normal weight and 25% overweight individuals.

Obese subjects at least 2½ times their ideal body weight were examined by Alexander (1963) and found to have a 50% incidence of hypertension to a slight or moderate degree. Direct intra-arterial blood pressure measurements were used. Ten percent of the individuals were severely hypertensive (blood pressure of 200/120 mmHg or greater).

One problem with correlations between blood pressure and body weight has been determining whether body weight itself is involved or the percentage of body fat. Kannel et al. (1967) observed that blood pressure was more strongly associated with body weight than body fat in human subjects. Meyer et al. (1969) also supported this concept when he pointed out that skinfold thickness and blood pressure are not as strongly correlated as are body weight and blood pressure. The extent to which overweight measures body fatness varies with body build.

The frequency with which blood pressure and overweight coexist suggests that there is a causal relationship between them. Chiang et al. (1969) concludes that weight gain constitutes one kind of environmental stress that brings a genetic predisposition toward hypertension into the open.

Diet and Essential Hypertension

Alteration in Fat, Protein and Carbohydrate

Kempner (1944 and 1948) pioneered interest in the relationship between diet and blood pressure when he fed a rice-fruit-sugar diet to 500 hypertensive patients. Blood pressure decreased in 64% of the individuals. Hatch et al. (1954) conducted one of the few experimental studies in which nutritional components of a diet were varied and tested for the effect on blood pressure. The study involved modification of Kempner's rice-fruit diet. Modification included the addition of protein, fat and vegetables to the

basic diet. No loss of beneficial effects was noted when 12 to 50 grams per day of low-sodium protein, 20 to 40 grams per day of fat and 200 grams per day of vegetables were added singly or in combination to the diet. The study was conducted with hospitalized hypertensive individuals. Interestingly enough, the addition of 3 grams per day of salt to the diets prevented any blood pressure lowering effect of the diet.

A 5% sucrose solution when given in conjunction with a 1% saline solution to rats by Hall and Hall (1966) produced a more dramatic rise in blood pressure than did a 5% glucose and 1% saline solution or a 1% saline solution alone. Hypertension appeared in the saline group by the 21st day of the experiment and eventually affected 60% of the rats in that group. In contrast, there was a 30% incidence of hypertension in the sucrose-saline group by the 14th day which increased to 90% at 21 days. None of the glucose-saline group were hypertensive by the 14th day however, some were in the prehypertensive range for blood pressure (140-149 mmHg). Eventually 60% of the latter group were hypertensive by the 21st day. Cardiac weights in each saline group of rats were greater than water controls but not different from each other. Kidney weight was increased in rats in the saline and sucrose-saline groups when compared to controls. Kidney weight was not increased in rats given the glucose-saline drinking solution.

Ahrens (1974) supports the view that sucrose stimulates

sodium retention and in turn hypertension, and that the effect of sucrose is mediated through the fructose molecule.

Salt and Essential Hypertension

Salt Appetite

The custom of adding salt to food is ancient. Not only has salt been used by humans for palatability and preservation of food but also for ritual and monetary exchange. It must be remembered however, that salt appetite, as it is commonly expressed, is acquired. An exception of course would include sodium deficiency (Blair-West et al. 1970) when an inherent requirement for salt would increase salt appetite or in the case of herbivorous animals desiring salt licks (Dahl 1972).

Actual metabolic requirements for sodium are small; 6-8 meq per day in the human infant and adult (Committee on Nutrition, American Academy of Pediatrics 1974). Average per capita sodium consumption in the adult in the United States is 150 to 200 meq per day (Committee on Nutrition, American Academy of Pediatrics 1974). In contrast, many people of the world living in the arctic, jungle and desert (primarily vegetarians) average 2 to 10 meq per day (Dahl 1972).

The ability of humans to adapt to a wide range of sodium intakes is due to the renal-endocrine system responsible for regulating body sodium. This is done by varying urinary sodium excretion according to sodium intake and

nonrenal sodium losses (Pitts 1970). The kidney and renin-angiotensin hormonal system are key factors in this physiological regulation and the regulation of blood pressure as well.

The human is not the only species which indulges in the use of large quantities of salt. When offered water and saline concurrently, rats show preference for saline in concentrations between 12 and 250 meq/L (0.0007 and 1.46%) (Blair-West et al. 1970). McConnell and Henkin (1973) found a preference for 0.30 M NaCl solutions in both spontaneously hypertensive female rats and normotensive controls using a 2-bottle preference test. The spontaneously hypertensive rats showed an increased preference for the salt solution as blood pressure increased to the hypertensive range throughout a 16 week period. The normotensive rats did not show an increase in preference with time.

Results similar to the preceding were secured by Hall et al. (1972). They found that Sprague-Dawley rats consumed much more of a 1% saline solution than Long-Evans rats. Hypertension developed in the former but not in the latter. Saline consumption increased as blood pressure increased.

Hypertensive humans have not been shown to have a higher mean taste threshold for salt than normotensives (Joossens 1973). Joossens believes that chronic and prolonged use of salt may effect the taste buds and thus impair the function of taste.

Human Hypertension

Evidence relating salt intake to human hypertension is indirect and derived from three sources; (1) therapeutic effects of salt restriction, (2) effects of salt elimination by diuretics, and (3) epidemiological correlations between salt intake and hypertension.

The blood pressure lowering effect of Kempner's rice-fruit diet has been shown by Hatch et al. (1954) to be due to the low sodium content of the diet. Dahl (1972) conducted a study using rats which further documented the effects of sodium restriction on blood pressure. Among rats, the level of salt consumed after hypertension had been established was critical, whether the disease had been produced by dietary salt or some other process. When rats were restricted in salt consumption the disease became more benign and life span was lengthened.

Human weight reduction studies conducted by Dahl et al. (1958) document that the decrease in blood pressure accompanying weight reduction was not the result of weight loss but the restriction of salt that usually accompanied caloric restriction.

Several diuretics used in the treatment of hypertension derive their therapeutic action from the elimination of salt in the urine (Joossens 1973). Hypertensive patients who have responded to diuretic drugs have responded equally as well to a salt restricted diet (Dustan et al. 1974).

Group correlations in different geographical areas of

the world have suggested a dose response between salt intake and prevalence of hypertension. A study of various primitive peoples in different areas of the world (Brazil, New Guinea and the Cook Islands) has indicated that blood pressure does not increase with age and hypertension does not develop as frequently as in some populations (Joossens 1973). In contrast, several population groups (United States, Japan and Sweden) consume consistently more salt than metabolically required and in turn exhibit elevated blood pressures (Dahl 1961, Joossens 1973). The northern Japanese for example, consume nearly twice as much salt as the southern Japanese (26 gm/day vs. 14 gm/day) resulting in an increased incidence of hypertension (40% in the north vs. 21% in the south) (Dahl 1961).

Salt in Experimental Hypertension

Indirect evidence is the only basis for a correlation between salt intake and hypertension in man since only a small number of studies have been conducted with human subjects. One such study was conducted by Gros and coworkers (1971) in which 6 grams of salt was supplemented to the normal salt intake of 7 untreated mildly hypertensive patients and 3 normal subjects. They found no edema or significant change in body weight or blood pressure in either group.

In the rat and other experimental animals a cause and effect relationship between salt intake and hypertension has been fairly well established. Lenel and associates (1948)

maintained 6-week old chicks on a 0.9% NaCl drinking solution and observed a higher blood pressure than in control chicks given tap water. When the drinking solution was changed to 1.2% NaCl, a greater increase in blood pressure was noted and associated with dehydration, diarrhea and subsequent loss of body weight. Blood pressure decreased when tap water was substituted.

Sapirstien et al. (1950) were successful in producing arterial hypertension in male rats by the substitution of a 2% NaCl solution for drinking water for a 6 week period. Autopsy revealed an hypertrophy of the heart and kidneys relative to body weight.

Self-sustaining hypertension has been induced in female rats by chronic feeding of salt at 8% by weight of the diet (Dahl et al. 1968). Rats were maintained on the high salt diet for one year during which time 80% became hypertensive. A four month follow-up period of salt restriction failed to lower blood pressure in two-thirds of the rats. The effect of excess salt ingestion on blood pressure was self-sustaining even after the original stimulus was removed.

The effect of salt on the development of hypertension in the spontaneously hypertensive rat has been examined frequently since these rats are considered to be the best model for investigating human essential hypertension (Aoki et al. 1972, Koletsky 1958). The relevance of heredity and environment to the development of hypertensive disease is easily demonstrated in these rats.

Aoki et al. (1972) found that spontaneously hypertensive rats developed hypertension (170 mmHg) whether fed a high or low salt diet. No difference in magnitude of blood pressure was noted between the two groups. However, when a 1% salt solution was substituted for drinking water and given to the rats already on a high salt diet the effect on blood pressure was marked. Hypertension developed earlier and reached a higher level (204 mmHg) in these rats. The same study indicated that growth rate and life span were significantly decreased in rats given the high salt diet and 1% saline.

Louis and coworkers (1969) employed a different strain of spontaneously hypertensive rat and observed hypertension with or without added salt in the diet. Gross increases in sodium intake (4% of the diet) increased the severity of the hypertension and depressed the growth rate as measured by body weight.

A greater sensitivity of young animals to excess sodium chloride has been documented by Dahl et al. (1967). The earlier and longer that rats received a diet containing 8% NaCl, the more severe was the hypertension that developed and the more difficult it was to reduce blood pressure with sodium restriction. Moderate to severe lesions in the kidneys of these rats were observed (Jaffe et al. 1970).

Sodium:Potassium Ratio in Experimental Hypertension

The ratio of sodium to potassium in the diet has been implicated as affecting the development of hypertensive

disease (Gros et al. 1971, Meneely et al. 1961). Meneely et al. (1961) conducted an interesting experiment in which rats were fed either a diet high in both sodium and potassium or high in sodium and low in potassium. After 8 weeks on the diets, blood pressure was higher in rats given the high sodium-high potassium diet than in rats fed the high sodium-low potassium diet. At 12 weeks blood pressures were equal in both groups. By 19 and 27 weeks the blood pressure of the high sodium-low potassium group was climbing and became significantly higher than pressure in the high sodium high potassium group. Meneely concluded that when the potassium level of the diet was sufficiently high to restore the Na:K ratio to one, significantly lower blood pressure could be maintained. Gros et al. (1971) failed to observe the same results in humans given a diet with a Na:K ratio of one.

Salt and Renal Mass in Experimental Hypertension

Supplemental salt intake in conjunction with a reduced kidney mass has been shown to accelerate the onset of hypertension in both rats and dogs (Molteni and Brownie 1972, Coleman and Guyton 1969, Langston et al. 1963, Douglas et al. 1964, Koletsky 1959).

Langston et al. (1963) removed 70% of the renal mass from dogs and observed a 30-40% increase in blood pressure within 48 hours after ingestion of a 0.9% NaCl solution. Blood pressure decreased to normal levels when tap water was resumed. Normal dogs with both kidneys intact were given

the 0.9% saline solution to drink; blood pressure increased by 10%. In addition, urinary sodium excretion doubled in the normal dogs indicating the intact kidney was necessary to handle the salt load.

Douglas et al. (1964) also removed 70% of the renal tissue of dogs maintained on a normal diet and tap water. Arterial blood pressure rose to hypertensive levels within one week after 1.2% saline was substituted for tap water. Blood volume increased initially but decreased to normal within 2 weeks. After a 35-day period of drinking 1.2% saline, tap water was again resumed and blood pressure returned to normal. Identical results were obtained by Coleman and Guyton (1969) in a similar experiment with dogs.

Mononephrectomy is another popular technique for reducing renal mass. When rats of several strains had one kidney surgically removed and were allowed free access to a 1% NaCl drinking solution; each strain was found to react differently (Molteni and Brownie 1972). Some of the strains became hypertensive and others did not. When hypertension was evident it was often accompanied by high plasma levels of sodium and renal and cardiovascular lesions. Other studies have noted these same lesions in the face of hypertension (Jaffe et al. 1970, Koletsky 1959).

Koletsky (1959) postulated that the greater the degree of renal ablation the more responsive the animal is to the hypertensive action of salt. Salt itself was more often effective in producing elevated blood pressure in Koletsky's studies than was a decrease in renal mass up to 50%.

Possible Mechanisms for Salt-Induced Hypertension

The usual sequence of events in salt-induced hypertensive vascular disease have been observed as; (1) increased periferal vascular tone, (2) hypertension, (3) generalized vascular disease, and (4) renal disease (nephrosclerosis) (Koletsky 1961). The exact cause of the vascular lesions is not clear but is felt to be a consequence of the high blood pressure itself producing wear and tear on the vascular wall resulting in structural damage (Brest and Moyer 1967). Another possibility is that the salt acts directly on the vascular wall (Koletsky 1961). Renal disease develops from severe vasoconstriction in the kidney (an autoregulatory response). Irreversible structural changes are associated with the narrowing of these renal vessels (Ledingham 1971).

Several theories have been postulated for the cause of the increased periferal vascular tone that eventually leads to hypertension. Among these are the blood volume and imbalance of electrolytes theories.

Blood Volume Theory

Coleman and Guyton (1969) and Douglas *et al.* (1964) have postulated that excess water and salt loading causes an increase in extracellular fluid volume and a resultant increase in blood volume. The increased blood volume presumably increases mean systolic blood pressure and in turn the venous return. The increased venous return, in accordance

with the Frank-Starling law of the heart, increases cardiac output. An increased cardiac output elevates arterial pressure which induces a baroreceptor reflex to reduce heart rate and total peripheral resistance. The validity of this theory depends on proof of long term autoregulation which is theorized to slowly increase total peripheral resistance or vascular tone and eventually reduce cardiac output. Both reduced cardiac output and increased vascular tone are evident artifacts in essential hypertension (Douglas et al. 1964, Ledingham 1971).

Imbalance of Electrolytes Theory

An imbalance of electrolytes in the vascular wall in salt-induced hypertension has been thought to increase vascular tone that eventually leads to hypertension (Koletsky 1958, 1961). The imbalance is presumably initiated by retention and intracellular accumulation of sodium and replacement of potassium by sodium. Vascular edema resulting from accumulation of sodium and water may decrease the diameter of the blood vessel wall enough to lead to increased resistance (Koletsky 1961).

Tobian (1960) referred to this phenomena as "water-logging" of the blood vessels. Mallov (1961) hypothesized the waterlogging was due to a change in vessel membrane permeability. The water and solutes imbibed by the vessel would increase vessel tension. Mallov observed that aortic strips from hypertensive rats, placed in hypertonic salt

solutions exhibited increased tension in contrast to behavior of strips from normotensive rats.

A second possible explanation for increased vascular tone found in hypertension is that an electrolyte imbalance causes cellular necrosis in the vessel. This is accomplished by altering the actomyosin complex or interfering with the energy producing mechanism in the vessel wall. Both are influenced by concentration of alkaline ions (Koletsky 1958).

Electrolytes in Tissues of Hypertensives

Total Body Sodium

Schackow and Dahl (1950) noted a lack of gross ccumulation of sodium or depletion of potassium in whole body samples of rats fed either a high salt (8% NaCl) or a low salt (0.35% NaCl) diet. Since the animals fed the high salt diet developed hypertension, the authors concluded that salt-induced hypertension did not occur as a result of salt retention in the tissues.

Greene and Sapirstien (1952) found total body sodium to be greatly increased in hypertensive rats while total body potassium remained at normal levels. Plasma sodium also remained within normal limits indicating to them that the sodium ion had either penetrated intracellularly or been deposited in bone.

Tissue Sodium

Several investigators (Schackow and Dahl 1950, Greene and Sapirstien 1952, Tobian 1960, Haight and Weller 1961) observed normal plasma sodium levels in rats made hypertensive with or without salt. Attention then focused on the electrolyte content of various individual tissues. In adult renal hypertensive rats the electrolyte composition of the brain, gut, heart, liver, skeletal muscle and skin was not significantly different from normal control rats (Tobian 1960).

Haight and Weller (1961) found no change in sodium, potassium or chloride content of skeletal or heart muscle of rats fed diets containing 2.8%, 5.6% and 8.4% sodium chloride despite the fact that blood pressure increased as the sodium chloride level increased.

Arteriolar tissue has been implicated as a possible storage site for sodium since the rise in arterial hypertension is mainly due to a narrowing of the arteriole lumen (Tobian 1960, Meneely et al. 1961, Haight and Weller 1961). Investigation has centered on the sodium and potassium content of the aorta. Principally because the aorta is more easily accessable than arteriolar tissue. Haight and Weller (1961) found a progressive increase in sodium and potassium content of rat aorta as dietary NaCl progressed from 2.8% to 8.4%.

Tobian (1960) injected rats with deoxycorticosterone and gave them a 1% NaCl drinking solution. Among rats in

which blood pressure remained normal, the sodium content of the aorta increased and potassium content decreased slightly. In contrast, the aorta of rats that developed hypertension had a larger increase in sodium concentration but in addition, developed an increase in potassium rather than a decrease. Tobian reported that the level of potassium in the aorta correlated better with blood pressure than the level of sodium. In both mild and severe hypertension a decrease in blood pressure was accompanied by a drop in potassium concentration of the aortic wall.

Na:K Ratio and Electrolyte Concentration

Meneely et al. (1961) studied three groups of rats fed a diet varying in the amount of sodium and potassium chloride. The control group received a diet containing 1.1% NaCl and 0.66% KCl; Na:K ratio was approximately one. The "high sodium" group were fed a diet containing 9.8% NaCl and 0.6% KCl; dietary ratio of sodium to potassium was thirteen. The "high sodium + potassium" group received a diet containing 9.8% NaCl and 6.1% KCl; dietary ratio of sodium to potassium was one, just as the control group. Rats fed the high sodium diet had more body sodium than did the controls or rats fed the high sodium + potassium. The high sodium group also had a higher blood pressure than the controls or those rats fed high sodium + potassium, in which case the Na:K ratio was restored to one.

Further support of the importance of the Na:K ratio in hypertension was offered by Stone et al. (1957). He

observed that rats fed a high salt diet developed hypertension, renal damage and a decreased life span. A longer life span and decreased incidence of hypertension resulted when dietary potassium was increased. Whole body analysis indicated a reduced sodium content in those rats given high levels of dietary potassium in conjunction with the high sodium when compared to rats given high amounts of sodium alone.

INTRODUCTION

Cardiovascular disease is augmented by the presence of elevated blood pressure. Concrete explanations for the common occurrence of hypertension are few and far between. Although hypertension is known to result from various diseases and stressful situations (Ledingham 1971), the majority of hypertensive individuals remain undiagnosed. Diet is one of the many factors that can be investigated in a search for elements contributing to hypertension.

The laboratory rat is a common model used in studies involving hypertension. The use of animals in such studies permits rigid dietary and environmental control. Heredity is an important factor in the development of hypertension (Ledingham 1971, Dahl 1961) and control of this variable is possible through use of laboratory animals.

A definate cause and effect relationship has been shown to exist between salt and hypertension in the laboratory rat (Dahl 1961, 1967, 1972, Sapirstien et al. 1950). The effect of diets of variable composition on blood pressure is less pronounced. Kempner (1944) decreased blood pressure in hypertensive humans through a high carbohydrate-low salt diet. Hatch et al. (1954) elucidated further by adding variable amounts of fat and protein to Kempner's diet and observing no change in the therapeutic effect of the diet.

Sucrose has been implicated as a causative factor in the development of hypertension (Ahrens 1974, Yudkin 1972) however, for lack of concrete verification, this remains merely speculation.

Studies by Hall and Hall (1966) have suggested that the form of carbohydrate in the diet is an important consideration when investigating hypertension. They found blood pressure to be higher in rats given a sucrose drinking solution as opposed to rats given a glucose solution.

Rats fed diets high in fat become obese (Schemmel et al. 1969, 1972) and exhibit higher blood pressures than "normal weight" rats of the same age (Beebe et al. 1974 abstract). Whether the elevated blood pressure is due to the obesity or the high fat diet has not been clarified. Obesity has been strongly correlated to hypertension in humans (Chiang et al. 1969); this has not been proven in the laboratory rat.

Since most blood pressure investigations have been conducted with rats fed a chow-type of diet, any investigation into the effect of dietary variation on blood pressure must also include a diet similar in composition to the chow-type diet for comparative reasons. Subsequently a grain diet was used as a control diet in the present study. The primary purpose of the study was to observe any change in blood pressure or pulse rate in rats fed diets which varied in the source of the primary energy component, i.e., fat, sucrose or cornstarch. The effect of a salt-diet interaction on these parameters was also of interest. In

an effort to explain the effect of diet and/or salt on blood pressure, growth rate, specific organ size and tissue electrolyte concentration were examined.

METHODS

Experimental Design

Experiment I

Sixty-four male Osborne Mendel rats were weaned between 21 and 24 days of age and divided into 4 groups of 16 animals each. Each group of rats was fed one of four different diets detailed in Tables 1 and 2.

The diets differed in the source of the primary energy component. A cornstarch diet was developed on the basis of 100 grams and contained 67 grams (67%) of cornstarch. Sucrose was the primary energy component of a second diet also based on 100 grams and containing 67 grams (67%) of sucrose. The sucrose ration contained 3.8 kcal./gm of diet based on 4, 4 and 9 kcal/gm for protein, sucrose and fat, respectively (Guthrie 1967). The kcal.:protein diet ratio as calculated from kcal consumed/gm of dietary protein eaten was 19.0 for the sucrose diet. The cornstarch ration contained 3.5 kcal./gm of diet based on 4, 3.6 and 9 kcal./gm for protein, cornstarch and fat, respectively (Bowes and Church 1963). The kcal.:protein diet ratio was 17.5 for the cornstarch diet.

A fat ration was developed which contained 51.8% fat in the form of vegetable shortening and contained 6.0 kcal./gm based on values of 4, 4 and 9 for protein, carbohydrate and

fat, respectively. The kcal.:protein ratio was 20.0. Previous work by Schemmel et al. (1970) indicated that rats fed a diet high in fat (60% Crisco) consumed fewer grams of diet (30% fewer) than rats fed a grain ration.

The fourth diet was a grain ration which contained 53.5% carbohydrate, as calculated by difference, predominantly from corn. The diet contained 3.4 kcal./gm (Schemmel et al. 1972) and a kcal.:protein ratio of 14.8. The grain ration was used to represent standard growth and development and to allow comparison with previous studies which dealt with hypertension in the rat. Much of the previous blood pressure investigations have been performed with rats fed a standard chow-type ration similar to the grain ration (Sapirstein et al. 1950, Dahl et al. 1967, Weller et al. unpublished data).

Each group of 16 rats was further divided into 2 groups of 8 animals each. One of the 2 subgroups was allowed to drink distilled, deionized water ad libitum, while the second group received a 2% sodium chloride (NaCl) in distilled deionized water solution ad libitum. Such a design facilitated comparison of dietary effects on blood pressure with and without sodium chloride interaction.

Weekly blood pressure and pulse rates were determined for each rat at the same time of day and on the same day each week. This procedure was followed for 10 weeks when the rats were sacrificed. Various parameters other than blood pressure and pulse rate were measured individually

for each animal. These included weekly food and fluid intakes throughout the course of the study, weekly body weight gain, body composition (percent fat, lean and moisture), specific organ weight (heart, right adrenal and right kidney) and sodium and potassium concentration of the serum, heart, kidney and bone (right hind femur).

Experiment II

A second study was undertaken in which 24 male Osborne Mendel rats were weaned at 21-24 days of age and divided into 4 groups of 6 rats each. They were fed the same diets used in Experiment I. The rats were allowed to drink distilled deionized water ad libitum for the first 3 days post-weaning. A 1% NaCl and water drinking solution was substituted for the distilled deionized water on day 4 and was continued for 9 weeks. This was followed by substitution of a 1.5% NaCl solution for an additional 9 weeks or until the experiment was terminated. Blood pressure, pulse rate, food and fluid intake and body weight were monitored weekly for individual animals and compared with data from Experiment I. Heart, right kidney and right adrenal gland weights were recorded at the time of sacrifice.

Experimental Conditions

All experimental animals were housed in a ventilated room where the temperature remained at $23 \pm 1^{\circ}$ C. Twelve hours of light and twelve hours of darkness were allowed in each

24 hour period. The animals were sheltered individually in screen bottomed metal cages 18 x 18 x 25 cm in size. Activity was neither promoted nor restrained. Food was available to the rat in porcelain cups and allowed ad libitum. A weekly record of food intake was kept for each rat throughout the experiment by weighing the food cup filled at the beginning of the week and subtracting from it the weight of the cup at the end of the week. Correction for spillage was made by retrieving as much of the spilled ration as possible from a paper towel placed beneath each cage and adding this amount to the cup weight at the end of the week.

Either distilled deionized water or a solution of sodium chloride and water were allowed to each animal ad libitum through a cylindrical 100 ml graduated water bottle. The drinking spout was ball-tipped to reduce leakage and facilitate weekly water intakes throughout the study. Very little leakage did occur ($< 1.0\%$) when the bottles were correctly placed in the holder. Minimum leakage was measured in 5 sample bottles over a period of 7 days. Leakage consisted of 2 ml/100ml water.

Blood Pressure and Pulse Rate Measurement

Weekly determinations of blood pressure and pulse rate were considered adequate in frequency for establishing any trends in either of these parameters over time.

Since emotional stress is known to temporarily raise

blood pressure and pulse rate (Ledingham 1971) the experimental conditions were standardized as much as possible. Determinations were carried out in the early hours of the day (8 a.m. to 1 p.m.) on the same day each week to minimize diurnal variation. All blood pressure and pulse rate determinations were made indirectly from the tail of the rat (Figure 1). This technique was employed because it allowed frequent determinations to be made without affecting the physical condition of the rat.

The rat was placed into a plexiglass restraining device designed to restrict movement. This was found most satisfactory in restraining the rat such that anesthetic was not necessary. The use of this device eliminated any influence anesthetic might have had on blood pressure and pulse rate.

Weanling animals were often too small to be completely restrained by the device used, in which case the unit was lined with foam rubber to accommodate the size of the animal. The unit plus the animal was then placed on a heating pad set such that surface temperature was maintained at $33 \pm 1^{\circ}$C. The heating pad was located on the floor of a large wooden box with a glass top[1]. This set-up was a modification of a more elaborate and expensive device offered by Narco Biosystems[2]. Each rat remained inside the restrainer in the box for 10 minutes prior to actual

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[1]Courtesy of Dr. Collings, Associate Chairman, Department of Physiology, Michigan State University.

[2]Life Science Instrumentation, Houston, Texas.

measurement of blood pressure and pulse rate. The 10 minute accommodation period was allowed for two reasons; (1) to allow the rat to relax and adjust to the new surroundings and (2) to raise the body temperature of the animal such that blood flowed freely through the tail. If the animal were not heated, blood flow through the tail would not be strong enough to be detected by the instrument used. A temperature range of 36.5-39.0° C produces dilation of the tail vessels of the rat without appreciably altering blood pressure (Geddes 1970).

Measuring blood pressure and pulse rate involved the use of a Desk Model Physiograph[3] with a plug-in module for recording these parameters. The tail of the animal was inserted through a rubber lined metal blood pressure cuff 1.1 cm in diameter and 3.3 cm in length. The cuff was positioned as close as possible to the point of attachment of the rat tail to the body. Because the tail of the rat is long and tapered, there is a pressure gradient along the tail. This necessitates that the cuff be placed at the root of the tail (Geddes 1970). Rubber tubing connected the cuff to the Electrosphygmograph Coupler[4] where a hand bulb for expanding the cuff was located.

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[3]The Desk Model Physiograph DMP-4B was obtained from Narco Biosystems Inc., Life Science Instrumentation, Houston, Texas.

[4]Type 7211 is a plug-in module of the Physiograph Solid State Channel Amplifier/Coupler System designed for the determination of indirect blood pressure. This coupler combines a pressure transducer and amplifier to produce single channel recordings of occluding cuff pressure and superimposed Korotkoff sounds of humans and animals.

A pneumatic pulse sensor was attached lightly with masking tape to the tail of the rat directly posterior to the blood pressure cuff. The sensor was attached through a short length of flexible tubing to a Pneumatic Pulse Transducer[5] which was connected via rubber tubing to the coupler. A single channel recording was produced to show pulsations of blood passing through the rat's tail. The number of pulses per minute was determined by multiplying the number of pulsations recorded in 3 seconds by 20. This number was established as the pulse rate.

Recording blood pressure required certain standard conditions. These are listed in Appendix A. Squeezing the hand bulb connected to the coupler expanded the pressure cuff lining and restricted blood flow through the tail. The single channel recording indicated the precise point of occluding cuff pressure by the absence of pulsations. Pressure in the hand bulb was slowly released until pulsations were again recorded. Blood pressure was determined by measuring the distance from the base line to the point where pulsations appeared on the recording paper. Each millimeter on the paper represented 4 mmHg blood pressure (Pressure range switch set at 0-100 mmHg; maximum sensitivity of 20 mmHg/cm). Since the Physiograph was capable of recording only the initial return of blood flow through the tail,

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[5]This is a sensitive piezoelectric transducer for detection of pulsatile volume changes.

diastolic blood pressure was not recorded by this technique. Recorded blood pressures represent systolic pressure only.

So as to establish accuracy and precision in blood pressure and pulse rate determinations a series of determinations were recorded consecutively at one minute intervals until at least 3 readings were identical to within 4 mmHg. Sometimes this would involve several measurements (10-12). Once the blood pressure and pulse rate were determined the rat was removed from the restraining device and returned to the appropriate cage.

Procedure for Sacrificing Rats and Removing Organs and Fat Depots

Each experimental animal was anesthetized with ether on the day of sacrifice. Approximately ¼ inch of the tail was removed at the tip with a razor blade. Blood was collected from the tail into a 3 ml test tube and spun in a centrifuge for 10 minutes at 3200 rpm[6]. The serum was removed into a second test tube, covered with parafilm and frozen. The animal was then killed with an overdose of ether. Immediately following death an incision was made down the midline of the rat on the ventral side. The right inguinal fat pad lying just under the skin was removed and the abdominal wall opened with an incision. The right testicular and perirenal-retroperitoneal fat pads were removed and each fat pad was weighed immediately to the third place.

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[6]International Clinical Centrifuge Model Cl.

The right kidney, right adrenal gland and the heart were removed and weighed to the nearest milligram immediately before being placed into individual self-sealing plastic bags and frozen. The right hind leg was removed, skinned, placed in a self-sealing plastic bag and frozen. Stomach, intestinal and cecal contents were removed before these organs and the remaining carcass were deposited into the glass jar, weighed and frozen.

Body Composition Analyses

Moisture Content

Each carcass was thawed to room temperature in the glass jar before autoclaving for 15 minutes under 15 pounds per square inch pressure (120°C) to soften the bones (Mickelsen and Anderson 1959). After autoclaving, the carcass and jar were reweighed and any water lost during autoclaving was replaced by the addition of distilled deionized water. The carcass was emptied into a 1 gallon Waring Commercial Blender[7] and an amount of deionized water approximating the carcass weight was used to rinse the jar and added to the blender. The mixture was blended for 3 minutes at high speed (15,500 rpm w/64 oz. water). Approximately 10 grams of the homogenate was weighed into a small dry, tared aluminum pan. Duplicate samples were taken from each carcass.

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[7]Model CB-6, Waring Products Service Center, New Hartford, Connecticut 06057.

Samples were placed in a drying oven for 24 hours at 70°C before being transferred to a vacuum oven. Following drying in the vacuum oven for 48 hours at 70°C the samples were cooled in a dessicator and weighed to a constant weight. Percentage moisture in the carcass was calculated using the following formulas:

$$\text{Percent dry weight of sample} = \frac{\text{dry weight of sample}}{\text{wet weight of sample}} \times 100$$

$$\text{Percent dry wt. of carcass} = \text{\% dry wt. of sample} \times \frac{\text{wt. carcass}^{8} + \text{wt. added water}}{\text{weight of carcass}^{8}}$$

$$\text{Percent moisture in carcass} = 100\% - \text{\% dry weight of carcass}$$

Body Fat and Lean

The 10 gram samples used for moisture determination were also used for determination of body fat. Samples were extracted with anhydrous ethyl ether for 6½ hours on a Goldfisch fat extraction apparatus. This length of time was found sufficient to remove fat from the samples since re-extraction of some samples failed to result in additional extracted fat. After extraction, the extraction flasks were dried in a 70°C oven for 15 minutes, cooled in a dessicator and weighed. The following calculations were used to determine body fat:

$$\text{Percent fat in sample} = \frac{\text{wt. of fat in sample}}{\text{dry wt. of sample}} \times 100$$

$$\text{Percent fat in carcass} = \frac{\text{\% fat in sample} \times \text{\% dry wt. of carcass}}{100}$$

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[8]Refers to weight of carcass before freezing.

Grams fat in carcass = % fat in carcass x carcass weight.

The total amount of lean body mass present in the rat carcass was determined by difference using the following calculations:

Percent lean body mass (LBM) = 100% - % fat in carcass

Grams of LBM = % LBM x carcass weight.

Preparation of Samples for Flame Emmission Spectrophotometry

Serum

All analyses were performed in duplicate. Frozen serum samples were thawed at room temperature before 0.1 ml of serum was diluted with 9.9 ml of distilled deionized water. The diluted serum was stored in an acid-washed[9] 100 ml polyethylene bottle until the sodium and potassium content of the sample was determined using flame emmission spectrophotometry.

Heart and Kidney

Both heart and kidney samples were treated identically for analyses of sodium and potassium. The organ was thawed at room temperature and homogenized on a Polytron

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[9]Acid wash consisted of 1 part concentrated hydrochloric acid to 2 parts distilled deionized water. Glassware and bottles were soaked in the wash for 20 minutes followed by a 20 minute soak in deionized water and then rinsed with deionized water 3 times (Ku, Pao. 1974 personal communication).

Homogenizer[10]. Homogenizer tubes were acid-washed. The homogenate contained 4 parts distilled deionized water to 1 part tissue. A 1 ml aliquot of homogenate was weighed into an acid washed phillips beaker for digestion. The digestion procedure was a modification of the wet digestion procedure for minerals found in The Official Methods of Analysis of the Association of Official Analytical Chemists (Horwitz 1970). The modification involved the use of smaller amounts of reagents and was therefore more economical and achieved the same results[11].

The sample of tissue was digested with 60 ml of concentrated nitric acid under a hood specifically designed for work with perchlorates[12]. When the digestion mixture was reduced to 3-5 ml the mixture was cooled before 7 ml of concentrated perchloric acid was added. Continued digestion required covering the beaker with a watch glass until the most reactive phase of digestion had passed. When the mixture was reduced to 3-5 ml the tared weight of the beaker was brought to 20 grams with distilled deionized water. The solution was transferred to an acid-washed polyethylene bottle for storage prior to analysis by flame emmission spectrophotometry.

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[10]Brinkmann Polytron Model PT 10-35, Brinkmann Instruments Inc., Cantiague, Westbury, N.Y. 11590

[11]Dr. Duane Ullrey, personal communication (1974).

[12]The hood was designed such that a continuous stream of water flowed down the back and roof of the hood. This allowed the perchlorate vapors to solubilize in the water and be carried away rather than accumulate in the hood where they are extremely explosive.

Bone

Analyses for sodium and potassium in the bone of the right hind leg required that the bone be perfectly free of any flesh. This required removal of the flesh by a technique more efficient and precise than mechanically possible. Therefore, a Dermested Beatle colony was used to eat away the flesh and leave clean bone[13]. Each bone was labeled with a cardboard tag attached to the joint and marked with waterproof ink. The entire process took approximately 2 weeks.

Once the bones were flesh free, the femur was removed, cut into several pieces and weighed. The bone fragments were wrapped in filter paper and inserted into a fat extraction tube. Fat was extracted from the bone for 6 hours so that mineral content could be expressed on a fat-free dry basis. The bone was extracted with absolute alcohol for 3 of the 6 hours on a Goldfisch fat extraction apparatus. Alcohol extraction was followed by extraction with anhydrous ethyl ether for the remaining 3 hours. The combined alcohol-ether extraction is more efficient for extracting total lipids than is ether alone (Joslyn 1970).

Following extraction, the bones were dried in a 110°F oven for 4 hours and ashed overnight (18 hours) in a muffle furnace set at 1200°F. The bone was ground to a fine powder

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[13]Obtained from Mr. Larry Bowdre, Assistant Museum Curator, Michigan State University.

with a mortar and pestle and transferred to an acid-washed polyethylene bottle. The powdered ash was dissolved with 5 ml of 6N hydrochloric acid and then diluted to 100 ml with deionized water and stored for analysis.

Diet Rations

Duplicate samples (0.25 gm each) of each ration were digested by the same wet ashing technique used for heart and kidney samples. Sodium and potassium contents of the rations were determined using flame emmission spectrophotometry.

Flame Emmission Spectrophotometry

All sodium and potassium determinations were performed using a Flame Emmission Spectrophotometer[14]. A fuel-oxidant mixture was used for flame emmission; air being the oxidant and acetylene the fuel. Wavelengths were set at 589 nm for sodium and 766.5 nm for potassium. Standards were evaluated on the same day as the samples. Experimental samples were often too concentrated and therefore were diluted to within the optimum detection range of the instrument. Concentration of elements in the samples was determined by the following calculation:

$$\text{ppm or ugm/ml} = \frac{(\text{concentration in ppm})\ (\text{final volume})}{\text{sample weight}}$$

[14]An Instrumentation Laboratories 453 Atomic Absorption Emmission Spectrophotometer.

Analysis of Data

Blood pressure and pulse rate were recorded weekly for each rat therefore the change in these values over time was of interest. Means and standard deviations were determined for all groups of rats after 1, 3, 5, 7, 9 and 10 weeks on the dietary regimes. Significance of diet, salt and time were determined by a 2-way repeated measures analysis (Sokal and Rohlf 1969). The repeated measures test observed trends in blood pressure and pulse rate throughout the experimental period. Actual calculation of the analysis was performed by the 3600 Controlled Data Corporation (CDC) computer, Michigan State University.

Students t-test (Sokal and Rohlf 1969) was used to compare final mean blood pressure and pulse rate. Data from animals fed the three semi-purified diets was compared to data from grain-fed rats. In addition, within diet groups data was compared with and without the salt drinking solution. Data from Experiment II was not compared statistically with data from Experiment I.

Means and standard deviations were calculated for body weight at weekly intervals. Final body weight of rats fed the various diets was compared with final body weight of grain-fed rats. Means and standard deviations were established for body composition data (percent fat, lean and moisture), heart, kidney and adrenal gland weights and compared via students t-test (Sokal and Rohlf 1969).

Sodium and potassium data for serum, bone, heart and kidney were analyzed with a students t-test after calculation of means and standard deviations were performed.

RESULTS AND DISCUSSION

Experiment I

Diet and Kcal Consumption

Rats that received the grain ration and distilled deionized water consumed a mean total of 1498±140 gm of diet over the 10 week feeding period from weaning to 13 weeks of age. Rats fed the high fat, high sucrose and high cornstarch diets in conjunction with distilled deionized water consumed 930±99, 1634±113 and 1605±161 gms of diet respectively for the same period of time (Table 3).

The caloric density of the diets varied due to variation in the energy value of the primary dietary components in each diet. The grain ration contained 3.4 kcal/gm (Table 2) while the high fat, high sucrose and high cornstarch rations contained a calculated 6.0, 3.8 and 3.5 kcal/gm respectively (Table 1). When food intakes were adjusted for differences in caloric value the gap in intake between rats fed the various diets diminished. Rats fed the grain diet consumed 5092±476 kcal in 10 weeks whereas rats fed either the high fat, high sucrose or high cornstarch diets consumed 5578±594, 6210±429 and 6099±612 kcal respectively (Table 3).

When rats received a 2% NaCl and water drinking solution instead of distilled deionized water, food consumption

decreased regardless of diet. Lenel et al. (1948) noted a similar decrease in food consumption in chicks fed a standard chow-type diet in conjunction with a 1% NaCl drinking solution.

The decrease in food consumption was least pronounced in rats fed the grain ration. They consumed 4059±622 kcal for the 10 week period which was a 20% reduction when compared to rats fed the grain ration and the distilled deionized water (Table 3). Rats fed the high cornstarch diet in conjunction with the 2% NaCl drinking solution consumed 4522±927 kcal (Table 3). This was a 26% decrease in energy consumption when compared to rats fed cornstarch and distilled deionized water.

Rats fed the high fat diet and the 2% NaCl drinking solution reduced caloric consumption to 3453±552 kcal (Table 3). This was a 38% decrease in energy intake however, this was still not as severe as the 58% decrease observed in rats fed the high sucrose diet in conjunction with the 2% NaCl solution. Rats in the latter group consumed 2605±894 kcal for the 10 week experimental period (Table 3).

Variation in the quantity of salt in a diet has been implicated as changing osmolarity of the diet (Miller and Czajka 1967). Hypertonic solutions in the stomach slow the rate of gastric emptying and contribute to a feeling of fullness. Whether the 2% NaCl drinking solution contributed to a high stomach osmolarity in those rats given the solution remains to be clarified. If such were the case the

rats would have experienced a slower rate of stomach emptying and the resultant full feeling may have led to a decreased desire for food.

Miller and Czajka (1967) force-fed hypertonic glucose solutions to infant rats and observed decreased rates of stomach emptying and increased mortality as the osmolarity of the solutions increased. Since rats fed the high sucrose diet and the 2% NaCl solution exhibited the most severe reduction in food consumed, perhaps the sucrose molecule was exerting an osmotic pressure in the stomach which was compounded by the osmolarity of the sodium chloride solution. Yudkin (1972) has found that sucrose contributes more than any other constituent to the osmotic pressure of food.

The smallest decrease in food consumption when drinking the 2% NaCl drinking solution occurred in rats fed the grain ration. This suggests that the grain diet somehow alleviates the feeling of fulness caused by the sodium chloride. Gastric emptying time may not be decreased in the grain-fed rats, on the contrary, it may be increased.

Fluid and Sodium Consumption

The intake of distilled deionized water by rats fed either grain, high fat, high sucrose or high cornstarch diets was 2306±360, 2466±468, 2120±195 and 2394±136 ml respectively for the 10 week experimental period (Table 3). Because the drinking solution did not contain sodium, the total sodium intake was based on the amount of sodium

consumed in the diet (See Appendix B for actual sample calculation). Rats fed the grain diet received 7.5±0.7 grams of sodium for the 10 week period. In contrast, rats fed the high fat, high sucrose and high cornstarch diets consumed 7.0±0.7, 8.2±0.6 and 8.0±0.8 grams respectively (Table 4).

When sodium values are converted to grams consumed per 100 grams body weight, again each group of rats received similar amounts of sodium; i.e., 2.0±0.2, 1.7±0.2, 1.9±0.2 and 2.1±0.3 gm/100 gm body weight for rats fed grain, high fat, high sucrose and high cornstarch diets respectively (Table 4).

Rats that received the 2% NaCl drinking solution increased fluid consumption by an average of 170% regardless of the type of diet. Rats fed grain in conjunction with the 2% NaCl solution consumed 6180±1480 ml of fluid for the 10 week experimental period (Table 4). In contrast, rats fed a diet of high fat, high sucrose or high cornstarch content consumed 6326±78, 6307±1117 and 6582±444 ml respectively (Table 4). Sapirstien et al. (1950) observed a similar increase in the fluid consumption of rats offered a 2% NaCl drinking solution. The hypertonicity of the solution appears to stimulate thirst. Pitts (1971) has suggested the existence of a hypothalamic integrative mechanism responding to changes in osmolarity by stimulating thirst.

Since rats receiving the 2% NaCl drinking solution consumed fewer grams of food than their dietary counterparts

given distilled deionized water, it follows that they received less sodium from the diet. When the total amount of sodium ingested for the 10 week period was calculated, the major portion was derived from the drinking solution. Rats fed the grain ration in conjunction with the 2% NaCl solution consumed a total of 53.9±11 gm of sodium (Table 4). Similar amounts of sodium were consumed by rats fed either the high fat, high sucrose or high cornstarch diet; i.e., 53.4±1.3, 52.4±7.5 and 57.0±3.7 gm respectively (Table 4). However, when the total sodium intake was converted to gm per 100 gm body weight, marked differences in sodium intake were observed. Grain-fed rats consumed 32.6±22.4 gm/100 gm body weight whereas high fat, high sucrose and high cornstarch fed rats consumed 26.5±0.9, 53.4±0 and 29.9±9.9 gm sodium/100 gm body weight respectively (Table 4).

Growth Rates

The body weight of rats given distilled deionized water increased linearly throughout the 10 week feeding period regardless of the diet fed (Figure 2). Rats fed the high sucrose and high fat rations maintained similar weight gains from the third experimental week until termination of the experiment. These were also the largest weight gains. At the time of sacrifice (13 weeks of age) the high sucrose-fed rats weighed 21% more than grain-fed rats, whereas rats fed the high fat diet weighed 9% more than grain-fed rats. Rats fed the high cornstarch diet gained weight less rapidly

throughout the 10 week period (Figure 2). At the time of sacrifice rats fed the high cornstarch diet had a body weight that was 5% greater than rats fed grain.

Winnie et al. (abstract 1973) observed that male Osborne Mendel rats fed a sucrose diet gained significantly more body weight than rats fed either high cornstarch or grain diets. Similarly, Schemmel et al. (1972) demonstrated that rats fed a diet high in fat maintained greater body weights than rats fed a grain ration. It was concluded from the study that the energy of the diet was easily transferred to body fat by the animals fed the high fat diet.

Rats that consumed the 2% NaCl drinking solution exhibited a marked decrease in rate of growth when compared to dietary counterparts given distilled deionized water. By the second experimental week all rats in the 2% NaCl group had lower mean body weights regardless of diet. As time progressed the difference in mean body weight between the two groups of rats became progressively larger until at 13 weeks of age those rats given the 2% NaCl solution weighed up to 50% less than rats given distilled deionized water to drink (Figure 3).

Animals given the 2% NaCl solution and fed the high sucrose diet maintained a slower rate of growth than rats fed either grain, high fat or high cornstarch diets with the 2% NaCl drinking solution (Figure 3). This smaller, less rapid gain in weight persisted throughout the experiment until at 13 weeks of age the high sucrose-fed rats weighed

34% less than those rats fed the grain ration. The growth rate of grain-fed rats appeared least retarded when compared to rats fed the high fat, high sucrose or high cornstarch diets (Figure 3) which probably reflected food intake.

Body weight of all groups of rats, regardless of drinking solution, appeared to reflect caloric consumption. An exception was the group of rats fed cornstarch. they consumed more calories than rats fed the high fat diet but maintained a lower mean body weight throughout the experiment. As in the case of Schemmel et al. (1972) this suggests that rats fed the high fat diet are probably more efficient in depositing body fat than are rats fed the high cornstarch diet.

As suggested earlier, rats given the 2% NaCl drinking solution may have experienced a "fullness" due to an osmotic effect which consequently reduced food consumption and in turn body weight. This effect was more pronounced in rats fed the high sucrose diet.

Blood Pressure

Blood pressure increased linearly during the first 4 weeks of observation in those rats which received distilled deionized water regardless of dietary treatment (Figure 4). Rats fed the high sucrose diet reacted slightly differently in that blood pressure increased markedly during the second week and then decreased in the third week (Figure 4).

Heymann and Salehar (1949) observed that blood pressure

increased rapidly with age in growing Long-Evans rats within the first 3 months and up to a weight of 200 grams. Although blood pressure of rats given distilled deionized water began to plateau after 7 weeks of age, this time period corresponded to a mean body weight of approximately 200 grams.

Blood pressure of all rats given the distilled deionized water remained in the normotensive range (< 140 mmHg) throughout the experiment. However, distinct patterns of changes in blood pressure over time were observed between diet groups. Grain-fed rats maintained the lowest blood pressures from week 2 until the experiment was terminated (Figure 4). In contrast the rats fed the high sucrose diet exhibited the highest weekly blood pressures with exception of weeks 3, 4 and 5. Rats fed the high cornstarch diet maintained blood pressures slightly below rats fed the high sucrose diet, with exception of weeks 3, 4 and 5 when blood pressure rose slightly above that of the high sucrose-fed rats (Figure 4). Rats fed the high fat diet developed blood pressures that were greater than grain-fed rats but less than blood pressures of rats fed either of the high carbohydrate diets.

Final blood pressure reflected the trend that had occurred for the entire 10 week experimental period. Grain-fed rats had a blood pressure of 109±13 mmHg in contrast to 114±14, 127±9 and 117±7 mmHg for rats fed the high fat, high sucrose or high cornstarch diets respectively (Table 5).

Results of Students t-test for comparison of mean blood pressures of rats fed the various diets indicate that rats fed the high sucrose diet had a significantly higher blood pressure than rats fed the grain ration ($P < .01$). Blood pressure of rats fed either the high fat or high cornstarch diets was not significantly different from blood pressure of grain-fed rats.

Animals given the 2% NaCl drinking solution exhibited an extremely labile blood pressure pattern with time (Figure 5). Rats fed the grain diet showed the greatest amount of stability and had the lowest blood pressure at the end of 10 weeks (127 ± 20 mmHg) (Table 5, Figure 5). Rats in this group remained normotensive throughout the experiment. Rats fed the high fat, high sucrose and high cornstarch diets exhibited a weekly fluctuation in blood pressure and eventually became hypertensive.

With exception of weeks 6 and 10, those rats fed the cornstarch diet maintained the highest blood pressure throughout the experiment (Figure 5). Blood pressure of these rats reached hypertensive levels during week 5 and peaked at 179 mmHg during week 8. This was followed by a progressive decline in blood pressure until week 10 when blood pressure was lower than that of rats fed the high sucrose diet (Figure 5).

Rats fed the high sucrose diet showed a progressive but variable rise in blood pressure throughout the experiment (Figure 5). In all reality blood pressure of these rats

reached the hypertensive level during week 6 but declined to normal in weeks 7, 8 and 9 before catapulting to 178 mmHg in week 10.

Meanwhile, rats fed the high fat diet exhibited considerable variability in blood pressure until week 7 when blood pressure began to rise at a steady rate (Figure 5). Blood pressure surpassed the hypertensive level during week 8 (154 mmHg) and continued to rise until the experiment was terminated.

Comparison of final mean blood pressure between different diet groups given the 2% NaCl drinking solution indicated that once again the rats fed the high sucrose diet attained a significantly higher blood pressure ($P < .02$) than grain-fed rats. High fat and high cornstarch-fed rats achieved higher blood pressures than grain-fed rats but they were not statistically different.

A Students t-test (Sokal and Rohlf 1969) was used to compare mean blood pressure between rats fed the same diet but given either the distilled deionized water or the 2% NaCl drinking solution. Results showed that in the group of rats fed the grain diet there was no significant difference in blood pressure regardless of which drinking solution was consumed. However, in each of the 3 remaining diets there was a significant difference in blood pressure between those rats given the distilled deionized water and those given the 2% NaCl solution. The difference was most significant in rats fed the high sucrose diet ($P < .001$). Animals fed the

high fat and high cornstarch diets developed blood pressures significantly different at the same level ($P<.01$).

Sapirstien et al. (1950) observed considerable variability in blood pressure of rats given a 2% NaCl drinking solution and fed a standard chow-type diet. He found that those rats which showed large amounts of variability in blood pressure were the rats which eventually became hypertensive. Perhaps this pattern of labile blood pressure is a prerequisite for eventual hypertension and therefore could be used as a warning signal of the ensuing disease. Since grain-fed rats given the 2% NaCl drinking solution showed the least amount of variability in blood pressure and were the only group receiving salt that remained normotensive, this suggestion seems noteworthy.

Reference should be made to the fact that data representing the high fat-fed and high sucrose-fed rats given the 2% NaCl drinking solution were obtained from 2 rats per group and should therefore be examined with caution. An unexplained high mortality rate occurred in rats under these two dietary regimes. However, this should not lessen the impact of the results since supportive data is explained in Experiment II (to be discussed later).

Dalderup et al. (1969) noted a decreased life span and early onset of glomerulonephritis in Wistar Albino rats when dietary sucrose was increased from 15% to 30% of the diet. Coupled with a hypertonic drinking solution, the effect of sucrose on life span seems more severe. Casual

observation of the rats that died suggests diarrhea may have been an important contributing factor. However, diarrhea was not evident in those rats fed the high fat diet in conjunction with the hypertonic salt solution.

The ability of excessive salt consumption to provoke hypertension in rats is well documented. The effect of a salt-diet interaction has not been as well established. Hall et al. (1966) observed higher blood pressures in rats given a 5% sucrose-1% NaCl drinking solution than in rats given a 5% glucose-1% NaCl solution. It has been suggested that the fructose molecule is the offender in such cases (Ahrens 1974).

Sucrose elevated blood pressure regardless if excess salt was consumed or not although the presence of salt accentuated the elevation. The cornstarch diet also elevated blood pressure when compared with the grain diet, but not to the same extent as sucrose. Such results would agree with data by Hall.

Rats fed the high fat diet developed a higher blood pressure than grain-fed rats. This is difficult to explain since rats are resistent to atherosclerosis (Bragdon and Mickelsen 1955) eliminating the possibility of atherosclerotic plaques narrowing the vessel lumen and subsequently elevating blood pressure. Obesity cannot be associated with the rise in blood pressure since no significant difference in body weight was observed between grain-fed and high fat-fed rats. When rats are fed a 60% hydrogenated fat

diet for 63 weeks a higher blood pressure is observed when compared to rats fed a grain diet for the same length of time (Beebe et al. abstract 1974). The possibility exists that the percentage of body fat might be better correlated to blood pressure than body weight.

Pulse Rate

Final mean pulse rates can be found in Table 5. Pulse rate trends with time are illustrated in Figures 6 and 7.

Mean pulse rates of rats given distilled deionized water followed a trend similar to that of blood pressure in the same diet group. Rats fed the high sucrose diet assumed the highest pulse rate throughout most of the experiment while rats fed the grain diet assumed the lowest. In contrast, rats fed the high cornstarch diet developed a pulse rate that was lower than rats fed the high sucrose diet but higher than rats fed the high fat diet.

Rats given the 2% NaCl drinking solution demonstrated as much variability in pulse rate with time as they had shown with blood pressure. Although considerable variation was observed between dietary groups at several points in time, there was no significant difference between pulse rates in the final experimental week.

According to Farris and Griffith (1949) normal pulse rate in the anesthetized rat is 300 beats/minute. Pulse rate can be influenced by several factors, one of which is temperature. When body temperature rises, heart rate

increases but blood vessels dilate and blood pressure remains unchanged or lowered (Ganong 1971). The fact that the rats were heated during pulse rate measurement may explain the overall higher pulse rates recorded that are in opposition to Farris and Griffiths' data. In addition, the rats were not anesthetized which may or may not have influenced pulse rate. Geddes (1950) has identified an average pulse rate of 420 beats/minute as normal in the unanesthetized rat.

Body Composition

Body fat and lean body mass expressed in grams and as a percentage of body weight are presented in Table 6. Total body water and the amount of water in lean tissue expressed as percent are also presented in Table 6.

Body Fat

The percentage of body fat in each group of rats, regardless of diet or drinking solution, reflected mean body weight. A linear increase in body fat with increasing body weight has been established in rats fed a high fat diet and in rats fed a grain diet up through a body weight of 450 gm (Schemmel et al. 1969).

Regardless of diet, rats given the 2% NaCl drinking solution had less body fat in terms of both total grams and percent of body weight than did rats given distilled deionized water. Body fat in rats of the former group ranged

from 10 gm (6% body weight) in rats fed the high sucrose diet to 22 gm (9% body weight) in rats fed the high cornstarch diet. Of the rats given distilled deionized water, body fat ranged from 30 gm (9% body weight) in grain-fed rats to 86 gm (24% body weight) in rats fed the high fat diet.

Individual comparison of diets and the effect on body fat accretion showed that grain-fed rats were least affected by the hypertonic drinking solution. Body fat in these rats was 9±3% when given distilled deionized water and 7±3% when given the 2% NaCl solution. Despite the fact that rats fed the grain diet in conjunction with the 2% NaCl solution consumed large amounts of sodium, they managed to maintain body weight and body fat closest to the weight and fat content observed in rats given the distilled deionized water.

Body fat in rats fed the high fat diet in conjunction with distilled deionized water was 24±4% as compared to 8±1% in high fat-fed rats given the 2% NaCl solution. Rats fed the high sucrose diet exhibited a similar reduction in body fat when given the 2% NaCl drinking solution; 6±5% in contrast to 20±5% when given the distilled deionized water. Rats fed the high cornstarch diet fared slightly better in that body fat was reduced to a smaller extent by the salt solution; from 15±4% in rats given the distilled deionized water to 9±7% in rats given the 2% NaCl solution.

Lean Body Mass

Since the percentage of body fat in rats given distilled deionized water was always greater than the percentage of body fat in rats given the 2% NaCl solution, it follows that the percentage of lean in the former group was always less than in the latter.

However, grams of body lean were higher in rats given distilled deionized water (Table 6). For instance, the bodies of rats fed the high fat diet and distilled deionized water contained 280±44 gm of lean tissue which represented only 76±4% of body weight. In contrast, rats fed the same diet but given the 2% NaCl drinking solution contained 163±15 gm of lean tissue which represented 92±1% of body weight.

All rats given distilled deionized water, regardless of diet, had comparable amounts of lean body mass when expressed as total grams rather than percent, i.e., 296±28, 280±44, 314±25 and 298±42 gm for rats fed grain, high fat, high sucrose and high cornstarch diets respectively (Table 6). The opposite affect occurred in rats given the 2% NaCl solution. Comparable amounts of lean body mass were observed in rats of this group when lean body mass was expressed as percent of body weight rather than total grams. The reason being, that rats given the 2% NaCl solution had less variability in body fat than rats given the distilled deionized water.

Body Water

Compared to rats given distilled deionized water, total body water was greater in those rats given the 2% NaCl drinking solution regardless of diet. Since rats in the latter group had a smaller percentage of body fat total body water would be expected to be greater. Progressive expansion of the fat stores of the body results in little increase in water content. Leanness is associated with a high body water fraction; obesity with a low (Pitts 1972).

For example, rats fed the high fat diet and distilled deionized water had a total mean body water content of 54±4% and a body fat content of 24±4%. In contrast, rats fed the same diet but given the 2% NaCl solution had a body water content of 67±4% and a fat content of 6±5%.

When total body water was expressed as percent of lean there was no difference in water content of the rats fed any diet or drinking solution. This indicated that tissues of animals given the hypertonic drinking solution were not retaining excessive quantities of fluid. Conversely, neither were the tissues dehydrated.

Organ Weights

Fresh weights of the heart, right kidney and right adrenal gland from individual rats can be found in Table 7. Weights are expressed as milligrams and milligrams per 100 grams of body weight.

Heart

Heart weight was greater in rats given distilled deionized water than in rats given the 2% NaCl drinking solution. Since the former group maintained higher body weights, they may be expected to also have heavier hearts in view of the constancy of the relationship between body weight and heart weight (Grommet, unpublished data). For example, rats fed the high sucrose diet in conjunction with distilled deionized water had the largest body weight and consequently the largest heart weight (1626±562 mgm). In contrast, rats fed the grain diet and distilled deionized water had the lowest body weight and the lowest heart weight (1130±110 mgm).

If rats fed the grain diet are of the same body weight as rats fed a high fat diet, it follows that heart weights are also equal (Grommet, unpublished data). This suggests that body weight is the critical factor influencing heart weight. However, heart weight was not simply a reflection of body weight when rats fed the high fat diet were compared to rats fed the high cornstarch diet. Rats given distilled deionized water and fed the high fat diet had a larger body weight than rats fed the high cornstarch diet, yet the rats fed the latter diet had heavier hearts, i.e., 1419±233 mgm for rats fed the high cornstarch diet vs. 1128±90 mgm in rats fed the high fat diet.

When fed the high fat ration rats show an increased efficiency for accretion of body fat (Schemmel *et al.* 1972). Perhaps calories are shunted towards fat stores

while muscular organs such as the heart are deprived of normal growth. On the other hand, since both sucrose and cornstarch diets produced the greatest hypertrophy of the heart, the carbohydrate diets themselves may influence cardiac hypertrophy in some way.

Hypertrophy of the heart was observed in all rats given the hypertonic drinking solution when heart weight was expressed as mgm/100 gm body weight. A cardiac hypertrophy of 25% above expected values, in addition to a consistent elevation of systolic blood pressure above 140 mmHg has been used as a criteria for evaluating hypertension in rats (Greene and Sapirstien 1952). If cardiac weight of grain-fed rats given distilled deionized water is used as the expected value, then rats fed the high fat, high sucrose and high cornstarch diets in conjunction with the 2% NaCl solution can be classified as hypertensive. Although grain-fed rats showed a cardiac hypertrophy above 25% when given the hypertonic solution, they did not experience an elevated blood pressure above 140 mmHg.

When examined on an individual basis of diet, rats fed the high sucrose diet and the 2% NaCl drinking solution exhibited the most severe cardiac hypertrophy. Hall and Hall (1966) also observed greater cardiac hypertrophy in rats given a 5% sucrose-1% NaCl drinking solution as opposed to rats given a 5% glucose-1% NaCl solution.

Kidney

Fresh weight of the right kidney was less in rats given the 2% NaCl drinking solution than in rats given distilled, deionized water (Table 7). This was probably a reflection of the larger body weights in the latter group. However, when kidney weight was converted to mgm/100gm body weight, all groups of rats given the 2% NaCl solution exhibited renal hypertrophy. Sapirstein et al. (1950) observed renal enlargement relative to body weight in rats given a 2% NaCl drinking solution and fed a chow ration.

When the effect of diet on kidney weight was examined, rats fed the high sucrose diet had the largest kidneys relative to body weight. This was true for those rats given either distilled deionized water or the 2% NaCl drinking solution. The right kidney of the latter group was 30% larger than the kidney taken from rats fed either high fat, grain or high cornstarch diets. If compared to kidneys of rats consuming the distilled deionized water , the size of the kidney from high sucrose-fed-2% NaCl solution group of rats was 100% larger than the kidneys from any group of rats regardless of diet.

Since absorption of glucose in the kidney requires work and therefore increases oxygen consumption in the kidney, it follows that renal hypertrophy may be a compensitory mechanism in the rats fed the high sucrose diet. However, this should be equally as true for the glucose derived from starch. Yet rats fed the high cornstarch diet did not

experience as much hypertrophy as did rats fed the high sucrose diet.

There is a possibility that the enlargement of the kidneys in rats fed the high sucrose diet may have been due to an accumulation of fat. This is based on the observation of Winnie et al. (abstract 1974) that rats fed a sucrose diet had a higher percentage of fat in the kidney than did rats fed cornstarch or grain. Overfeeding has been shown to produce renal lesions and renal hypertrophy in rats (Kennedy 1960). It is possible that fat stored in the kidney has pathological effects on renal tissue.

Adrenal Gland

Right adrenal glands taken from rats given the 2% NaCl drinking solution weighed less than adrenal glands from rats given the distilled deionized water with exception of rats fed the high fat diet. When these weights were converted to mgm/100gm body weight those rats given the 2% NaCl solution showed adrenal hypertrophy ranging from 25% to 114% above dietary counterparts given distilled deionized water. Rats fed the grain diet and the 2% NaCl solution had adrenal glands that were 25% heavier than those receiving distilled deionized water. Rats fed either of the high carbohydrate diets increased adrenal size by 50% over dietary counterparts given distilled deionized water. Adrenal hypertrophy was most severe in rats fed the high fat diet and the 2% NaCl solution. Adrenal weight increased 114% above high fat-fed rats given distilled deionized water.

It has been suggested that salt hypertension is produced through a synergism between large quantities of salt and endogenous secretions of the adrenal cortex (Sapirstein et al. 1950). Constant stimulation of the adrenal gland may cause the gland to hypertrophy in rats given the 2% NaCl solution. On the other hand, if the renin-angiotensin-aldosterone system were reacting normally to a salt load, the gland may exhibit atrophy due to lack of stimulation by angiotensin (Berman 1973).

Sodium and Potassium Concentrations

Serum

Normal serum levels of sodium and potassium were present in rats fed the grain ration and given distilled, deionized water to drink (Tables 8 and 9). However, all remaining serum sodium values were below normal regardless of diet or drinking solution. Conversely, serum potassium concentrations fluctuated between normal and above normal. Rats fed the high sucrose diet showed exceptionally high potassium values of 8.0 ± 3.4 and 10.6 ± 0.4 meq/L for rats given distilled deionized water and the 2% NaCl solution respectively. Such values are beyond the physiological maximum for normality of 6.0 meq/L.

Several investigators (Schackow and Dahl 1950, Greene and Sapirstein 1952, Meneeley et al. 1961, Haight and Weller 1961) have failed to show a change in serum sodium or potassium concentration in rats which ingested large quantities of sodium chloride.

Heart

Normal sodium concentration in the heart of the rat is approximately 39 meq/gm of fresh tissue (Spector 1956). Most of the rats maintained normal sodium concentration in the heart regardless of diet or drinking solution. Rats fed either the high sucrose or high cornstarch diet and given the 2% NaCl solution were the exception in that these rats had identical sodium values of 48 meq/gm which were 23% higher than the expected value (Table 8).

Results of several observations has suggested that concentration of sodium does not increase in the hearts of rats given excess NaCl in the diet or drinking solution (Meyer et al. 1950, Meneeley et al. 1961, Haight and Weller 1961). Concentration of sodium in the hearts of animals fed the different diets did not reflect sodium intake and therefore would support the work of previous investigators.

Potassium concentration in the heart of rats from each experimental diet group was slightly higher than the expected value of 84 meq/gm of fresh heart tissue (Spector 1956) (Table 9). Any differences in potassiim concentration between rats fed the various diets or given either drinking solution were slight and nonsignificant. Haight and Weller (1961) failed to observe any change in heart potassium concentration in rats given excess dietary sodium.

Concentration of sodium and potassium in the aorta has been shown to increase linearly with increasing quantities of NaCl in the diet of rats (Tobian 1960, Haight and Weller

1961). In addition, concentration of these electrolytes was greater in hypertensive rats and became progressively greater as blood pressure increased.

Kidney

Since normal concentrations of sodium and potassium in the kidney of rats could not be obtained from the literature values for the rabbit were used as a guideline for expected values. Sodium concentration in the kidney of rats was slightly greater than expected from data for the rabbit which denotes 109 meq sodium/gm of fresh tissue as being normal (Spector 1956).

Sodium concentrations in the kidney of rats given distilled deionized water were similar regardless of diet fed; i.e., 124±10, 122±10, 125±18 and 126±16 meq/gm of fresh kidney for rats fed grain, high fat, high sucrose or high cornstarch diets respectively (Table 8). In contrast, rats given the 2% NaCl solution exhibited a marked increase in the amount of sodium retained in the kidney. Rats fed the grain, high fat and high sucrose diets increased kidney sodium concentration to 152±39, 165±49 and 165±12 meq/gm of fresh tissue respectively (Table 8). Each of these values was significantly greater ($P < .05$) than values for rats given the distilled deionized water and fed the same diet. Sodium concentration of the kidney in rats fed the cornstarch diet and the 2% NaCl solution was 197±47 meq/gm (Table 8). This was a highly significant increase ($P < .01$)

when compared to concentration in kidneys of rats given the distilled deionized water.

Interestingly, concentration of sodium in the kidney appears to reflect the total amount of sodium consumed for the 10 week period. Rats that consumed distilled deionized water consumed approximately equal amounts of sodium (Table 4) and subsequently had similar concentrations of sodium in the kidney (Table 8). Rats given the 2% NaCl solution ingested similar amounts of sodium for the 10 week period with exception of rats fed the high cornstarch diet which consumed slightly but not significantly more sodium (Table 4). Concentration of sodium in the kidneys of these rats exhibited the same pattern. Rats fed the high fat, grain and high sucrose diets had similar concentrations of sodium in the kidney whereas rats fed the high cornstarch diet had slightly but not significantly more sodium in the kidney (Table 8).

Meyer et al. (1950) failed to observe any change in the concentration of sodium in the kidney when rats were fed 5% sodium in the diet. These results suggest that the administration of sodium through the drinking solution has more marked effects on the retention of sodium in the kidney. The capacity of the kidney to excrete sodium in the urine may be impaired.

Potassium concentrations observed in rats appear similar to the normal value for rabbits of 84 meq/gm fresh kidney (Spector 1956). Concentration of potassium in

kidneys of all groups of rats regardless of diet or drinking solution were not significantly different from each other with exception of rats fed the high fat diet and the 2% NaCl solution. Potassium concentration in these rats was 26% greater than the expected value of 84 meq/gm (Table 9). Meyer et al. (1950) observed a significant increase in the concentration of potassium in the kidneys from rats fed a high sodium diet but he failed to offer an explanation.

Bone

For lack of a value for bone sodium and potassium in the rat, values from horse femur were used as a guideline. Concentrations in the rat femur were within the same range as for the horse femur, i.e., 130-169 meq sodium/gm dry fat-free bone (DFF) and 4.0-5.0 meq potassium/gm dry fat-free bone (Spector 1956).

Sodium concentration of the right hind femur of rats given distilled deionized water and fed the grain, high fat, high sucrose and high cornstarch diets was 139±21, 147±56, 147±25 and 156±37 meq sodium/gm DFF bone respectively (Table 8). These values were not significantly different from each other which suggests no effect of diet on sodium concentration in the bone.

Sodium concentration in the femur of rats given the 2% NaCl solution were markedly higher than concentration in rats given distilled deionized water. Sodium concentration ranged from 50-136% above values obtained in the latter group.

These results confirm speculation of Greene and Sapirstein (1952) that bone is a labile storage site for sodium.

Sodium intake relative to body weight was similar in rats given the 2% NaCl solution and fed either grain, high fat or high cornstarch diets (Table 4). Concentration of sodium in the femur of these rats was also relatively equivalent (Table 8). Only 1 femur sample could be attained from rats fed the sucrose diet and given the 2% NaCl drinking solution. This rat consumed the largest amount of sodium relative to body weight (53.4 gm/100 gm body weight) and in turn had the highest concentration of sodium in the femur.

If concentration of sodium in the bone increases proportionately with sodium intake then perhaps the bone is a labile storage site for sodium. Variable quantities of sodium could be stored by the bone which would play an important role in maintaining constant plasma and tissue levels of sodium.

Regardless of diet, the concentration of potassium in the femur of rats given distilled deionized water was relatively the same and near the normal value of 5.0 meq potassium/gm DFF bone (Table 9). In contrast rats given the 2% NaCl solution had lower than normal potassium values with exception of the high sucrose-fed rat (Table 9).

Potassium concentration may be expected to decrease in the bone if it is assumed that sodium ions replace potassium ions when the rat is on a high sodium diet. On the other

hand, potassium ions may move into the bone in an attempt to maintain a constant sodium:potassium ratio. This could explain the high potassium content in the femur of the rat fed the sucrose diet.

EXPERIMENT II

A second study was conducted in an effort to accumulate additional data concerned with the effect of diet and salt consumption on blood pressure. Since the 2% NaCl drinking solution appeared toxic to rats fed the high sucrose and high fat diets, it was hypothesized that a reduction in concentration of the solution might decrease mortality and provide comparable results.

The same diets used in Experiment I were used in the second study. The experimental time period was extended to 18 weeks post weaning. During the first 9 weeks rats were given a 1% NaCl solution followed by a second 9 weeks of a 1.5% NaCl solution. During the sixth week of the experiment, a 2% drinking solution was substituted for the 1% solution; within 24 hours 2 high sucrose and 1 high fat-fed rat died. This necessitated a return to the 1% NaCl drinking solution.

Diet, Kcal, and Sodium Consumption

Total grams of diet consumed in 18 weeks for rats fed grain, high fat, high sucrose and high cornstarch diets were 2875±99, 1684±86, 2577±160 and 3168±270 respectively (Table 10). The lower intake in rats fed the high fat diet was due to the fact that this diet was calorically more dense than the other diets. When grams of diet were converted to kcal., food consumption equalized between diet groups. Rats fed

the grain, high fat, high sucrose and high cornstarch diets consumed 9775±335, 10,104±56, 9793±608 and 12,038±1026 kcal. respectively for the 18 week experimental period (Table 10).

Total fluid intake was relatively equal for all rats regardless of diet; i.e., 8563±600, 8969±1026, 10,469±1214 and 9055±1470 ml. for rats fed grain, high fat, high sucrose and high cornstarch diets respectively (Table 10). Rats fed the high sucrose diet drank slightly more of the salt solution than did rats fed the three remaining diets but the differences were not significant. Once again if sucrose produced hyperosmolarity, thirst would be stimulated as a compensitory mechanism to return osmolarity to normal.

Because the rats fed the high sucrose diet ingested larger amounts of the salt solution, total sodium intake was greatest in this group of rats. However, there was no statistical difference in total sodium intake between rats consuming the different diets. Rats fed the grain, high fat, high sucrose and high cornstarch diets consumed 56.7±5.8, 56.4±9.9, 66.7±15.9 and 61.5± 15.1 gm of sodium respectively (Table 10). Total sodium intake represents sodium from both the diet and drinking solution. Sodium from the drinking solution constituted proportionately more of the total intake than did any specific diet (Table 11).

If total sodium intake was expressed as gm sodium/100 gm body weight the same pattern occurred since body weights were relatively equal among rats regardless of diet. Rats fed the grain, high fat, high sucrose and high cornstarch

diets consumed 12.9±1.3, 11.7±2.1, 15.2±3.4 and 14.3±3.4 gm sodium/100 gm body weight (Table 11).

Growth Rate

All rats gained body weight at the same rate over the 18 week period regardless of diet (Figure 8). The severely depressed rate of growth observed in rats given the 2% NaCl drinking solution in Experiment I was not evident in rats given the 1-1.5% NaCl solution. In fact, if growth rates of rats in the first 10 weeks of Experiment II are compared with rats of Experiment I the growth curves are nearly identical with exception of rats fed the high sucrose and high fat diets (Figures 8 and 2). Slightly less than expected growth rates for rats fed either of these diets was evident from the sixth experimental week. However, the depressed growth rate was not as severe as in rats given the 2% NaCl solution in Experiment I (Figure 3).

Pulse Rate

Mean pulse rates followed a trend that was similar to pulse rates observed in rats given distilled deionized water in Experiment I. Rats fed the grain, high fat, and high cornstarch diets experienced a rapid decline in pulse rate occurring in the first 3 experimental weeks. Pulse rate then proceeded to level off with a certain degree of weekly variability (Figure 9). Rats fed the high sucrose diet, maintained the highest pulse rate throughout the experiment.

A multivariate analysis of variance of pulse rate from rats of Experiment I and II through the first 10 experimental

weeks indicated an effect of both diet and time on pulse rate (Table 12). The greatest effect of time on pulse rate was seen post-weaning when pulse rate decreased in the first 3 weeks. The effect of diet on pulse rate was probably due to the higher values exhibited by rats fed the high sucrose diet. The physiological significance of a higher than normal pulse rate would be manifested in a greater work load for the heart (Opie 1965).

Blood Pressure

Mean systolic blood pressure increased linearly and at similar rates in all rats during the first 3 experimental weeks regardless of the diet fed (Figure 10). During the remaining period of time, blood pressure was more labile, however, not to the same extent as in rats fed the 2% NaCl solution in Experiment I.

During the 4th week, rats fed the high sucrose diet exhibited a greater blood pressure than rats fed either grain, high fat, or high cornstarch diets. Rats fed the high sucrose diet maintained the highest blood pressures throughout the remaining 14 weeks of the experiment (Figure 10). Animals fed the grain, high fat, and high cornstarch diets eventually achieved approximately the same blood pressure as rats fed the high sucrose diet. There was no signigicant difference in blood pressure of the different groups in the 18th week.

By the 18th week rats fed the high sucrose diet developed a mean systolic blood pressure of 140 mmHg which was the

only value in the hypertensive range. Blood pressure was climbing in all rats when the experiment was terminated which may indicate that definate hypertension may have eventually evolved with time.

When data from both Experiments I and II are examined for equivalent lengths of time (first 10 weeks), a multivariate analysis of variance indicates an effect of a diet-salt interaction on blood pressure (Table 13). The effect of salt is obviated in the fact that blood pressure was elevated in all animals that received a NaCl drinking solution. The effect of diet appears to center around the high sucrose ration. Rats fed the high sucrose diet exhibited the highest mean blood pressure regardless if a salt solution was consumed or not (Figure 10).

It is apparant that the mechanism responsible for the development of hypertension is accelerated by the presence of the high sucrose diet. Furthermore, the addition of sodium chloride further augmented the blood pressure raising effect of sucrose.

TABLE 1.--Composition of Rations.

Ingredients	Diets (%) Fat	Cornstarch	Sucrose
Protein[a]	29.9	20.0	20.0
Fat: Crisco	51.8	--	--
Corn oil	--	3.0	3.0
Carbohydrate: Sucrose	2.2	--	67.7
Cornstarch	2.2	67.7	--
Minerals[b]	7.5	5.0	5.0
Sodium[c]	0.68	0.62	0.50
Potassium[c]	0.59	0.52	0.49
Fiber[d]	4.5	3.0	3.0
Vitamins[e]	1.5	1.0	1.0
Dl-Methionine[f]	0.37	0.25	0.25
kcal/gm[g]	6.0	3.8	3.8

[a]Casein, purchased from General Biochemicals, Chagrin Falls, Ohio.

[b]Rogers and Harper salt mix, purchased from General Biochemicals, Chagrin Falls, Ohio.

[c]Values obtained from flame emmission spectrophotometry, Model 453, Instrumentation Laboratories.

[d]Cellulose type, purchased from General Biochemicals, Chagrin Falls, Ohio.

[e]A.O.A.C. Vitamin mix, purchased from General Biochemical Chagrin Falls, Ohio. Supplied the following (gm/kgm diet): p-aminobenzoic acid, 0.10; B_{12}, (0.1% in mannitol), 0.03; biotin, 0.0004; calcium pantothenate, 0.04; choline, free base, 2.0; folic acid, 0.002; l-inositol, 0.10; menadione, 0.005; Niacin, 0.04; pyridoxine HCl, 0.04; riboflavin, 0.008; thiamine HCl, 0.005; dextrose, anhydrous, q.s.; (units/kgm) Vitamin A, 20,000.00; Vitamin D_2, 2,000.00; Vitamin E acetate, 100.00.

[f]Purchased from General Biochemicals, Chagrin Falls, Ohio.

[g]Values used for calculating kcalories were 4, 4, and 9 for 1 gm of protein, carbohydrate and fat, respectively.

TABLE 2.--Composition of Grain Ration.

Ingredients[a]	Percent
Protein	23.4
Fat	3.0
Carbohydrate (by difference)	53.5
Fiber	3.8
Ash	6.3
Moisture	10.0
kilocalories/gm[b]	3.4

[a]The grain diet contained (in %): ground corn, 60.7; soybean meal (50% protein), 28.0; alfalfa meal (17% protein), 2.0; fish meal (62.5% protein), 2.5; dried whey (67% lactose), 2.5; limestone (38% calcium), 1.6; dicalcium phosphate (18.5% P, 23.4% Ca), 1.75; and iodized salt, 0.5. The following were also added: (in mg/kgm feed) Mn, 121; Fe, 95; Cu, 7; Zn, 4; I_2, 4; Co, 2; choline chloride, 400; Ca pantothenate, 6; riboflavin, 3; niacin, 33; menadione, 2; Dl-Methionine, 500; penicillin, 2; streptomycin, 8; arsanilic acid, 968; (in ug/kgm feed) vitamin B_{12}, 7; and (in IU/kgm feed) vitamin A, 8010; vitamin D_2, 750; and vitamin E, 5.

[b]Schemmel, R. et al. 1972

TABLE 3.--Cumulative diet and fluid intakes of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Kcal/gm diet	Diet intake[a]		Fluid intake[a]
		gm	kcal	ml
Grain (8)[b]	3.4	1498±140[c]	5092±476[c]	2306±360[c]
Grain + 2% NaCl (7)	3.4	1194±183	4059±622	6180±1480
Fat (8)	6.0	930±99	5578±594	2466±468
Fat + 2% NaCl (2)	6.0	576±92	3453±552	6326±78
Sucrose (8)	3.8	1634±113	6210±429	2120±195
Sucrose + 2% NaCl (2)	3.8	686±238	2605±894	6307±1117
Cornstarch (8)	3.8	1605±161	6099±612	2394±136
Cornstarch + 2% NaCl (4)	3.8	1190±244	4522±927	6582±444

[a]Accumulated over a 10 week period.

[b]Number of rats in each group.

[c]Mean ± standard deviation.

TABLE 4.--Cumulative sodium intake of male Osborne Mendel rats fed either a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Na from food[a] gm	Na from water[a] gm	Total Sodium[a] gm	Total Sodium[a] gm/100gm BW.
Grain (8)[b]	7.5±0.7[c]	--	7.5±0.7[c]	2.0±0.2[c]
Grain + 2% NaCl (7)	6.0±0.7	48.0±11.5	53.9±11.0	32.9±23.4
Fat (8)	7.0±0.7	--	7.0±0.7	1.7±0.2
Fat + 2% NaCl (2)	4.3±0.7	49.1±0.6	53.4±1.3	26.5±0.9
Sucrose (8)	8.2±0.6	--	8.2±0.6	1.9±0.2
Sucrose + 2% NaCl (2)	3.4±1.2	48.9±8.6	52.4±7.5	53.4±0
Cornstarch (8)	8.0±0.8	--	8.0±0.8	2.1±0.3
Cornstarch + 2% NaCl (4)	6.0±1.2	51.1±.35	57.0±3.7	29.9±9.9

[a]Cumulative intake over a 10 week period.

[b]Number of animals in each group.

[c]Mean ± standard deviation.

TABLE 5.--Final blood pressure and pulse rate of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Blood Pressure mmHg	Pulse Rate bpm
Grain (8)[a]	109±14[b]	395±61[b]
Grain + 2% NaCl (7)	127±20	357±71
Fat (8)	114±13	385±64
Fat + 2% NaCl (2)	154±8[c]	410±46
Sucrose (8)	127±9	435±46
Sucrose + 2% NaCl (2)	178±14[c]	310±71
Cornstarch (8)	117±7	433±48
Cornstarch + 2% NaCl (4)	150±20[c]	395±77

[a]Number of rats in each group.

[b]Mean ± standard deviation.

[c]Considered hypertensive (>140 mmHg).

TABLE 6.--Body weight and body composition of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Body wt.[a]	Body Fat		Body Lean		Body Water	
	gm	gm	%	gm	%	total %	lean %
Grain (8)[b]	372±29[c]	30±8[c]	9±3[c]	296±28[c]	91±3[c]	66±2[c]	73±2[c]
Grain + 2% NaCl (7)	232±113	15±11	7±3	187±94	93±3	67±2	73±2
Fat (8)	405±58	86±20	24±4	280±44	76±4	54±4	71±5
Fat + 2% NaCl (2)	202±11	15±0	8±1	163±15	92±1	64±1	70±1
Sucrose (7)	451±43	83±29	20±5	314±25	80±5	57±3	71±4
Sucrose + 2% NaCl (2)	158±71	10±11	6±5	128±56	94±5	67±4	71±4
Cornstarch (8)	390±55	53±20	15±4	298±42	85±4	61±3	72±4
Cornstarch + 2% NaCl (4)	210±79	22±25	9±7	167±63	91±7	65±4	71±4

[a]Live body weight not adjusted for organs removed.

[b]Refers to number of rats in each group.

[c]Mean ± standard deviation.

TABLE 7.--Heart, right kidney and right adrenal gland weights for male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Heart[a]		Kidney[a]		Adrenal Gland[a]	
	mgm	mgm/100gm body wt.	mgm	mgm/100gm body wt.	mgm	mgm/100gm body wt.
Grain (8)[b]	1130±110	310±30	1260±180	340±40	30.0±8.0	8.0±2.0
Grain + 2% NaCl (7)	919±264	449±138	1104±316	534±145	24.1±7.6	10.9±5.1
Fat (8)	1128±90	320±50	1390±170	340±30	30.0±7.0	7.0±1.0
Fat + 2% NaCl (2)	810±170	399±62	1098±60	543±0.6	31.1±3.1	15.4±0.7
Sucrose (7)	1626±562	363±131	1678±494	373±111	32.6±13.4	11.6±3.4
Sucrose + 2% NaCl (2)	825±269	538±71	1043±223	698±171	17.7±1.5	12.2±4.5
Cornstarch (8)	1419±233	373±103	1334±276	347±90	29.3±10.3	7.5±2.4
Cornstarch + 2% NaCl (4)	905±212	449±69	1118±226	559±103	24.9±10.5	11.7±2.3

[a]Mean ± standard deviation.

[b]Refers to number of rats in each group.

TABLE 8.--Sodium content of serum, heart, right kidney and right hind femur of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Serum meq/L	Heart meq/gm[a]	Kidney meq/gm[a]	Femur meq/gm[b]
Grain (8)[c]	163±59[d]	40±5[d]	124±10[d]	139±21[d]
Grain + 2% NaCl (7)	108±16	43±9	152±39	218±123
Fat (8)	121±26	38±2	122±10	147±56
Fat + 2% NaCl (2)	122±14	42±3	165±49	236±226
Sucrose (7)	123±42	41±4	125±18	147±25
Sucrose + 2% NaCl (2)	123±31	48±7	165±12	495±0[e]
Cornstarch (8)	102±22	38±5	126±16	156±37
Cornstarch + 2% NaCl (4)	89±12	48±9	197±47	234±57

[a]Expressed as fresh tissue.

[b]Expressed as fat free dry tissue (DFF).

[c]Number of rats in each group.

[d]Mean ± standard deviation.

[e]Represents data from 1 rat only.

TABLE 9.--Potassium content of serum, heart, right kidney and right hind femur of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a drinking solution of distilled deionized water or 2% NaCl for 10 weeks.

Treatment	Serum meq/L	Heart meq/gm[a]	Kidney meq/gm[a]	Femur meq/gm[b]
Grain (8)[c]	5.0±1.6[d]	93±12[d]	82±8[d]	5.1±1.3[d]
Grain + 2% NaCl (7)	5.3±1.1	90±10	88±12	3.5±2.1
Fat (8)	5.3±1.0	88±7	87±9	5.6±1.0
Fat + 2% NaCl (2)	6.3±1.1	98±15	105±0	2.0±0.4
Sucrose (7)	8.0±3.4	96±4	82±10	5.0±0.5
Sucrose + 2% NaCl (2)	10.6±0.4	87±4	89±1	6.6±0[e]
Cornstarch (8)	4.0±0.8	90±10	79±18	5.4±0.5
Cornstarch + 2% NaCl (4)	5.8±1.9	92±8	81±9	3.5±1.1

[a]Expressed as fresh tissue.

[b]Expressed as fat free dry tissue (DFF).

[c]Number of rats in each group.

[d]Mean ± standard deviation

[e]Represents dat from 1 rat only.

TABLE 10.--Cumulative dietary and fluid intake of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a 1-1.5% NaCl drinking solution for 18 weeks.

Treatment	Food Intake[a]		Fluid Intake[a]
	gm	kcal	ml
Grain (6)[b]	2875±99[c]	9775±335[c]	8563±335[c]
Fat (5)	1684±86	10104±516	8969±1026
Sucrose (5)	2577±160	9793±608	10469±1214
Cornstarch (6)	3168±270	12038±1026	9055±1470

[a]Cumulative for 18 weeks.

[b]Number of rats in each group.

[c]Mean ± standard deviation.

TABLE 11.--Cumulative sodium intake of male Osborne Mendel rats fed a grain, high fat, high sucrose or high cornstarch diet in conjunction with a 1-1.5% NaCl drinking solution for 18 weeks.

Treatment	Sodium from food[a] gm	Sodium from fluid[a] gm	Total Sodium[a] gm	Total Sodium[a] gm/100gm body wt.
Grain (6)[b]	14.4±0.5[c]	42.3±5.8[c]	56.7±5.8[c]	12.9±1.3[c]
Fat (5)	12.6±0.6	45.7±5.7	56.4±9.9	11.7±2.1
Sucrose (5)	12.9±0.8	54.0±15.5	66.7±15.9	15.2±3.6
Cornstarch (6)	15.8±1.4	45.6±7.8	61.5±15.1	14.3±3.4

[a]Cumulative for 18 weeks.

[b]Number of rats in each group.

[c]Mean ± standard deviation.

TABLE 12.--Multivariate Analyses of Variance for Repeated Measures--effect of time, salt and diet on pulse rate.

	F Statistic	Degrees of Freedom	Probability P<
Repeated measures effect (time)	16.3794	4 & 57	.0001***
Interaction (time x diet)	1.6058	12 & 151	.0954
Interaction (time x salt)	0.8967	8 & 114	.5219
Interaction (time x salt x diet)	0.9396	24 & 200	.5483
Diet effect	3.6389	3 & 60	.0177*
Salt effect	0.8232	2 & 60	.4440
Interaction (diet x salt)	1.2096	6 & 60	.3140

TABLE 13.--Multivariate Analyses of Variance for Repeated Measures--effect of time, salt and diet on blood pressure.

	F Statistic	Degrees of Freedom	Probability P<
Repeated measures effect (time)	93.9569	4 & 57	.0001***
Interaction (time x diet)	0.9651	12 & 151	.4847
Interaction (time x salt)	5.0220	8 & 114	.0001***
Interaction (time x salt x diet)	1.1109	24 & 200	.3344
Diet effect	5.9498	3 & 60	.0013***
Salt effect	33.0763	2 & 60	.0001***
Interaction (diet x salt)	4.4961	6 & 60	.0009***

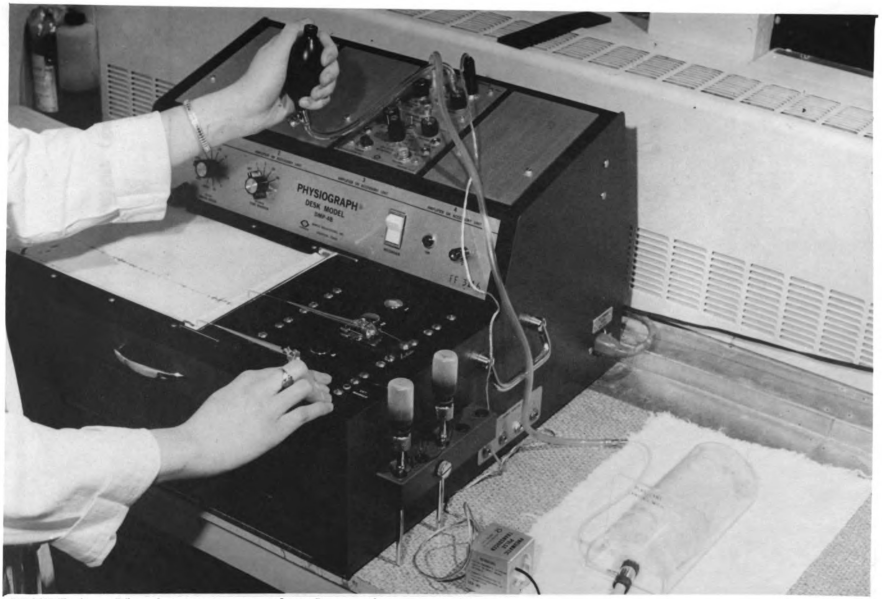

FIGURE 1.--Blood pressure and pulse rate apparatus.

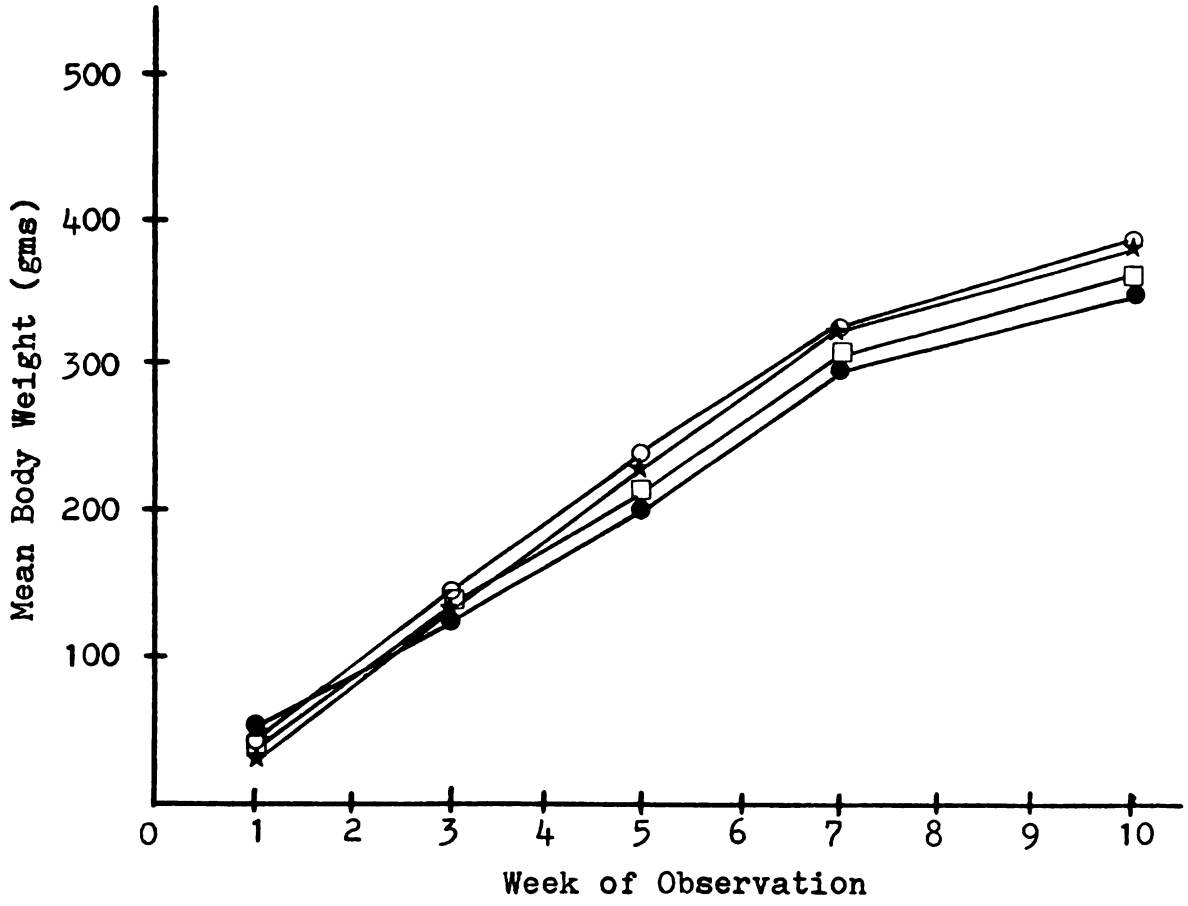

FIGURE 2.--Mean body weight of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○) or high cornstarch (□) diet in conjunction with a drinking solution of distilled deionized water for 10 weeks post-weaning.

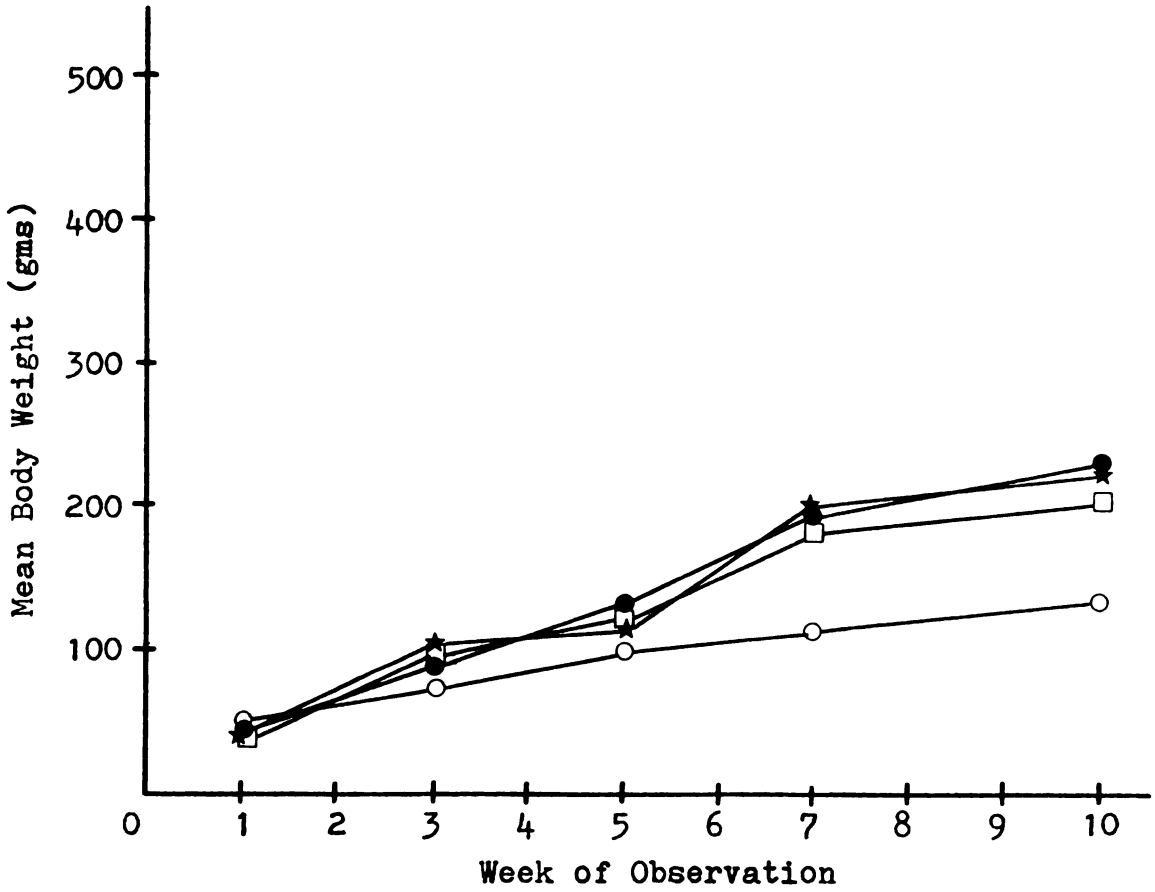

FIGURE 3.--Mean body weight of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○) or high cornstarch (□) diet in conjunction with a 2% NaCl drinking solution for 10 weeks post-weaning.

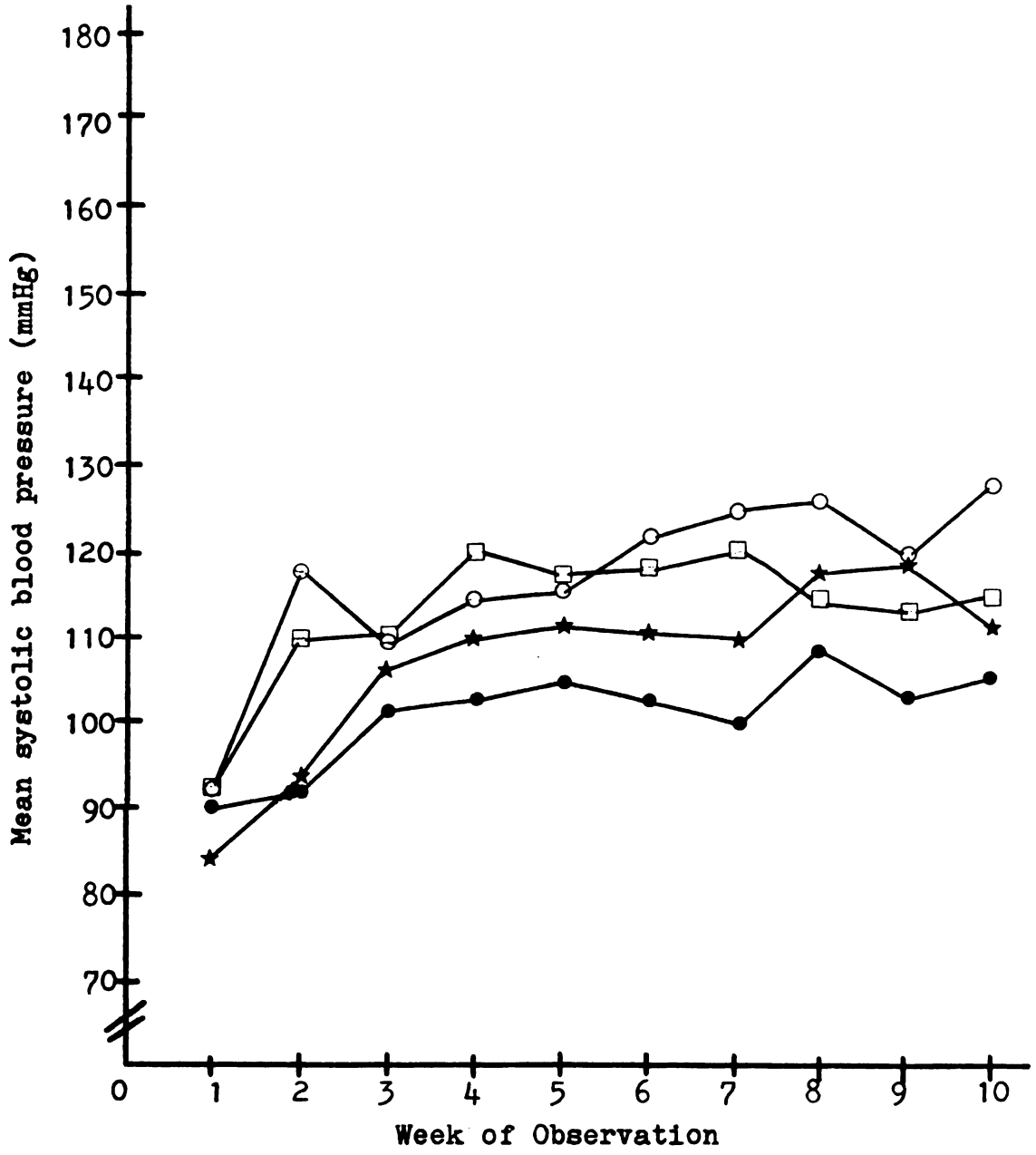

FIGURE 4.--Mean systolic blood pressure of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○) or high cornstarch (□) diet in conjunction with a drinking solution of distilled deionized water for 10 weeks post-weaning.

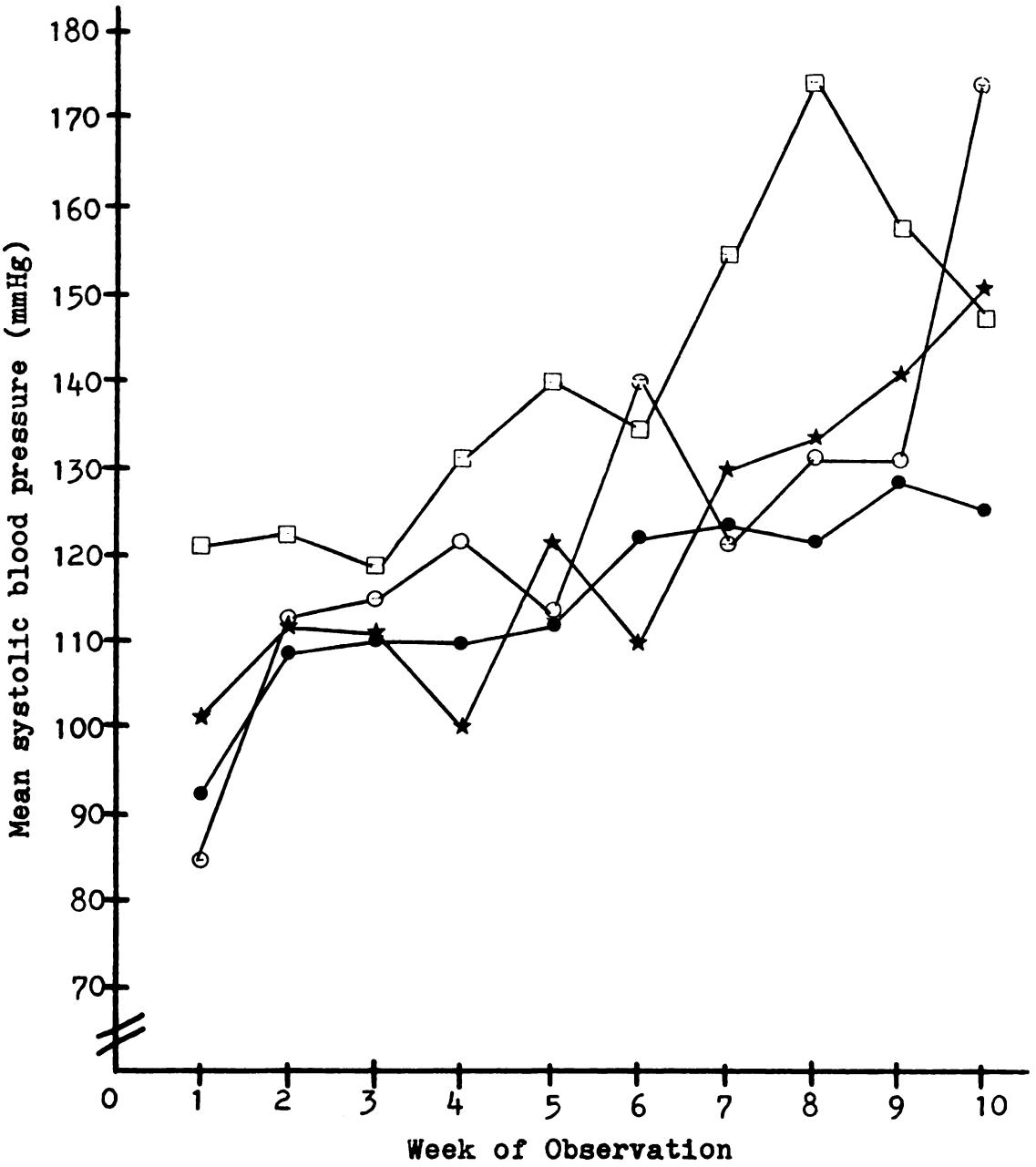

FIGURE 5.--Mean systolic blood pressure of male Osborne Mendel rats fed a grain (●), high fat (✱), high sucrose (ʘ) or high cornstarch (□) diet in conjunction with a 2% NaCl drinking solution for 10 weeks post-weaning.

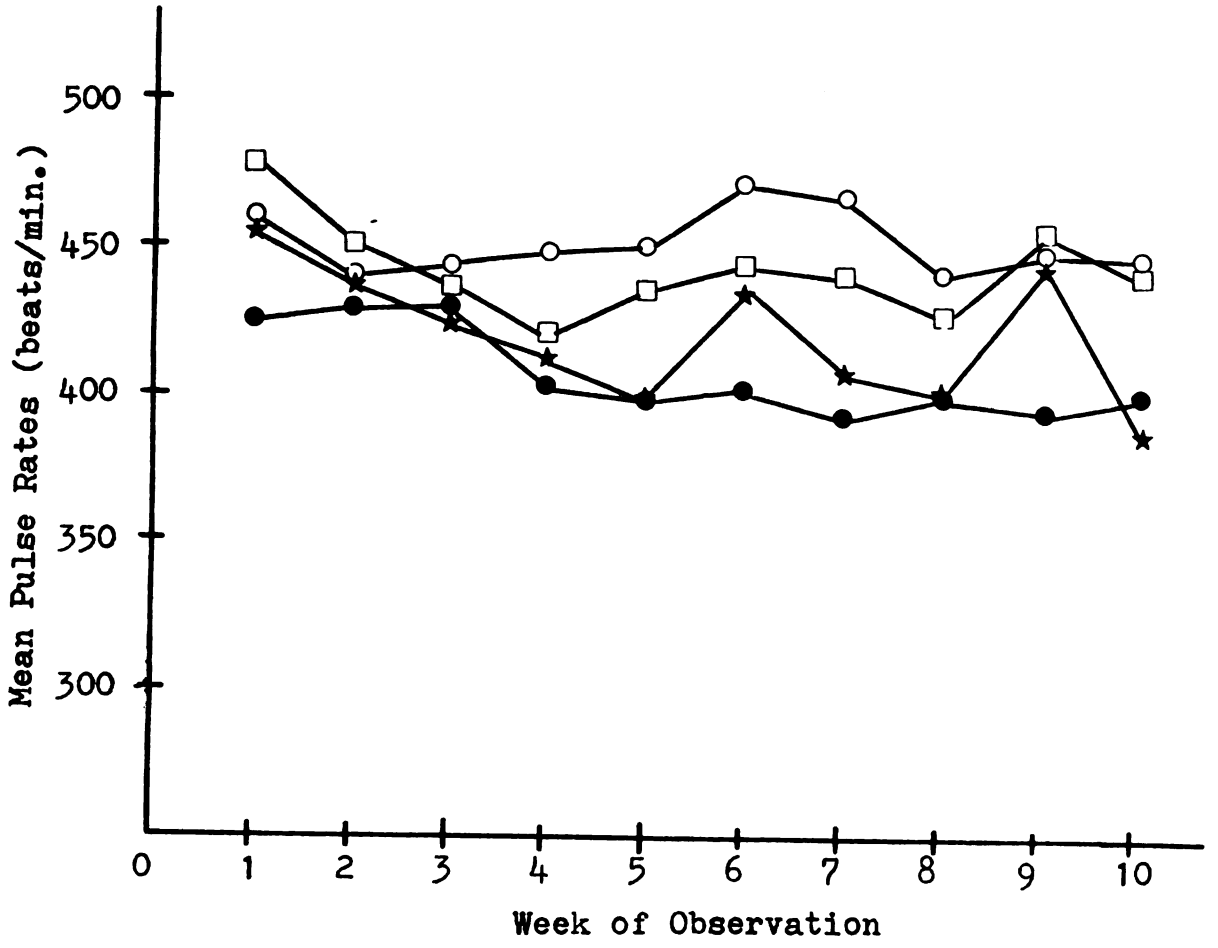

FIGURE 6.--Mean pulse rates of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○) or high cornstarch (□) diet in conjunction with a drinking solution of distilled deionized water for 10 weeks post-weaning.

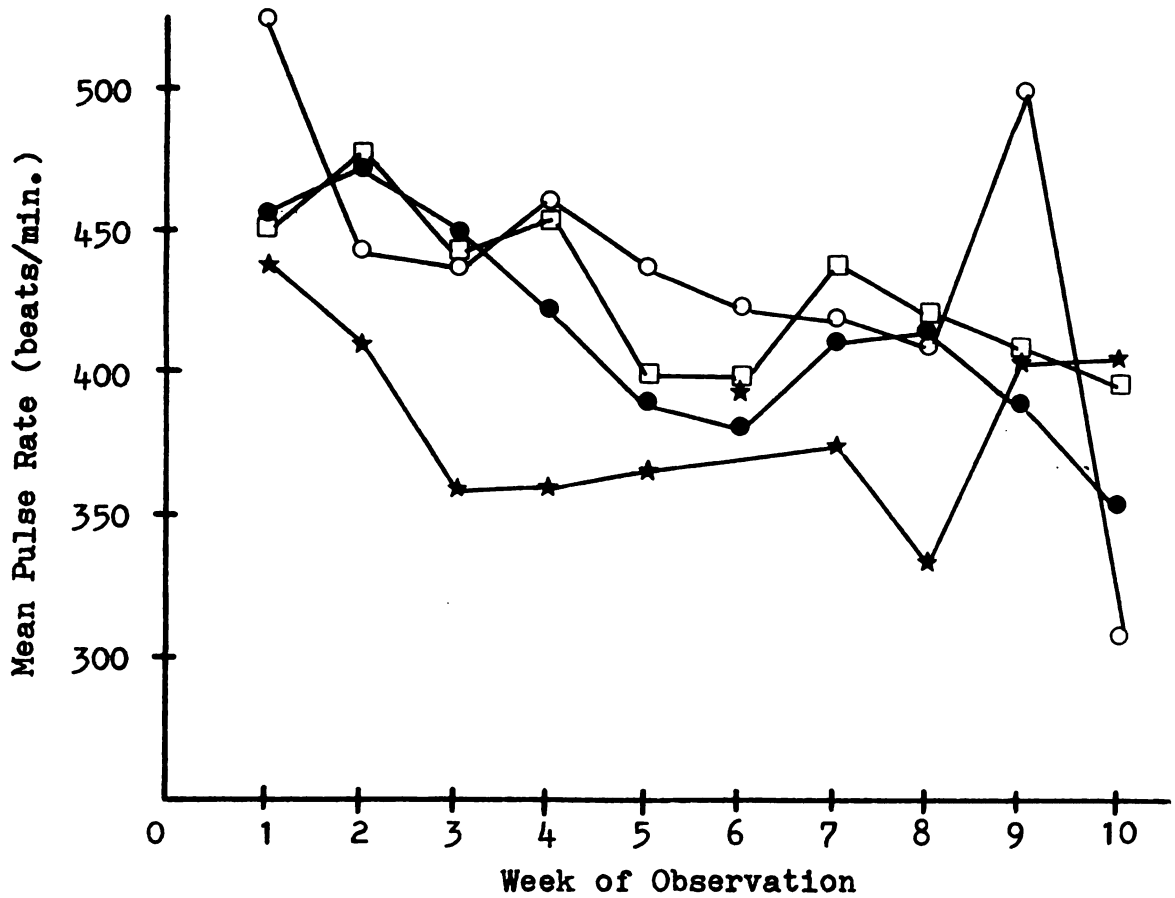

FIGURE 7.--Mean pulse rates of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○) or high cornstarch (□) diet in conjunction with a 2% NaCl drinking solution for 10 weeks post-weaning.

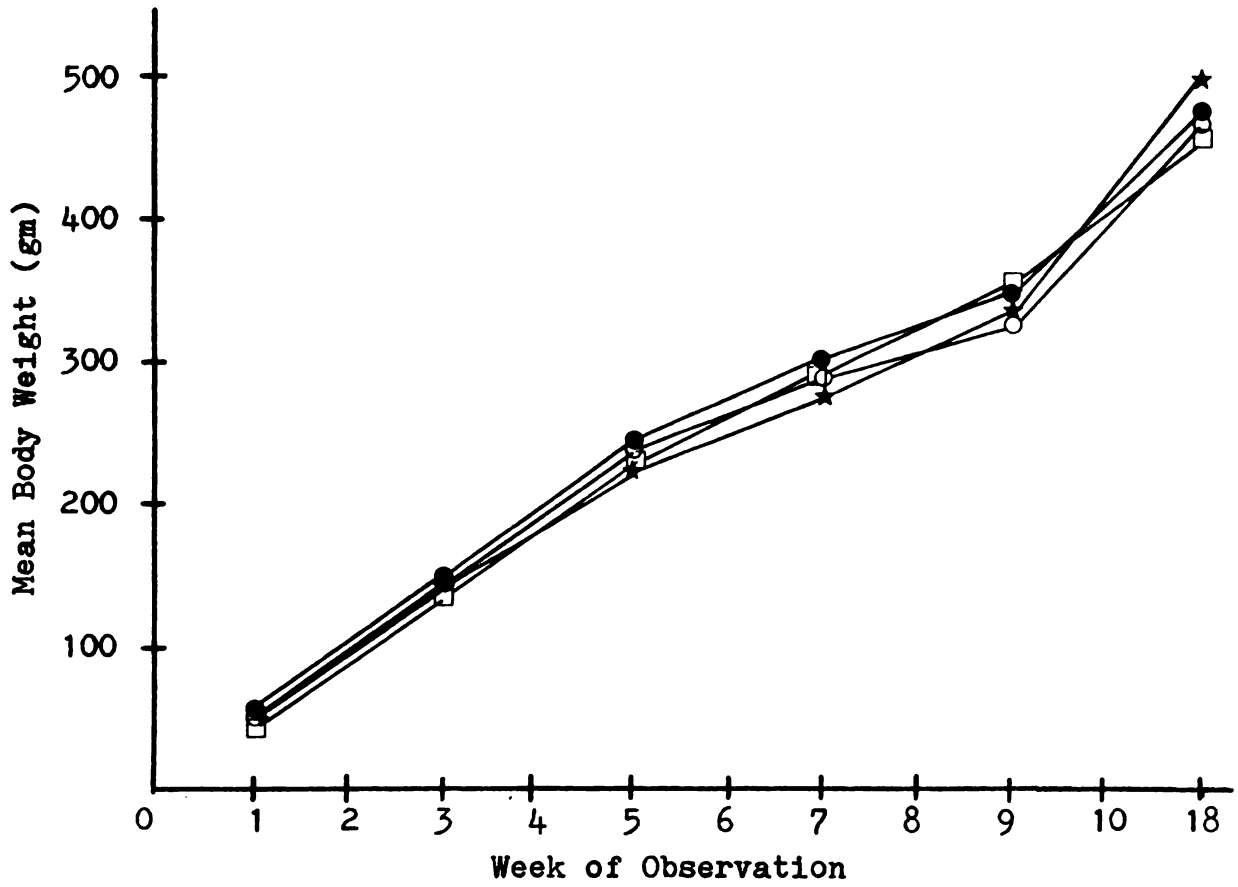

FIGURE 8.--Mean body weight of male Osborne Mendel rats fed a grain (●), high fat (✱), high sucrose (○) or high cornstarch (□) diet in conjunction with a 1-1.5% NaCl drinking solution for 18 weeks post-weaning.

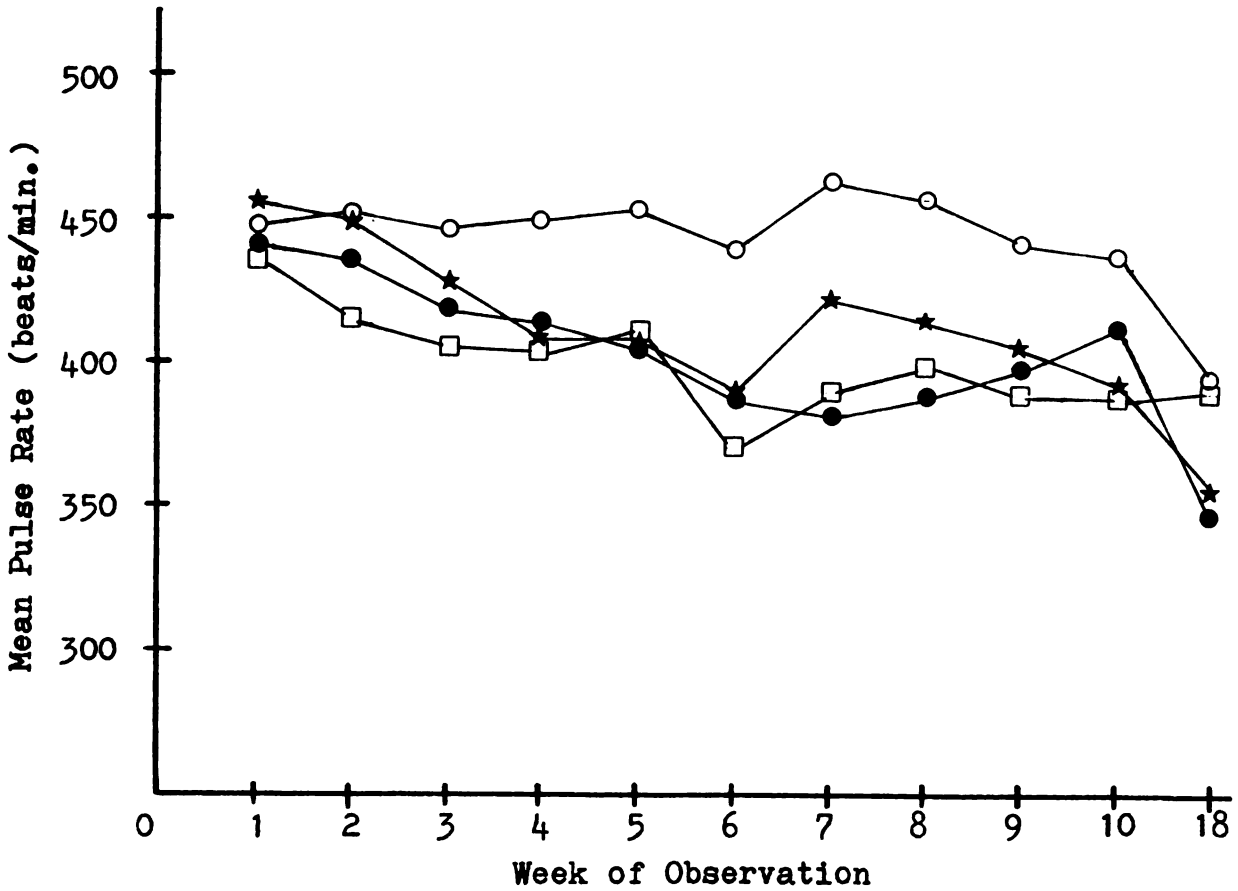

FIGURE 9.--Mean pulse rates of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○) or high cornstarch (□) diet in conjunction with a 1-1.5% NaCl drinking solution for 18 weeks post-weaning.

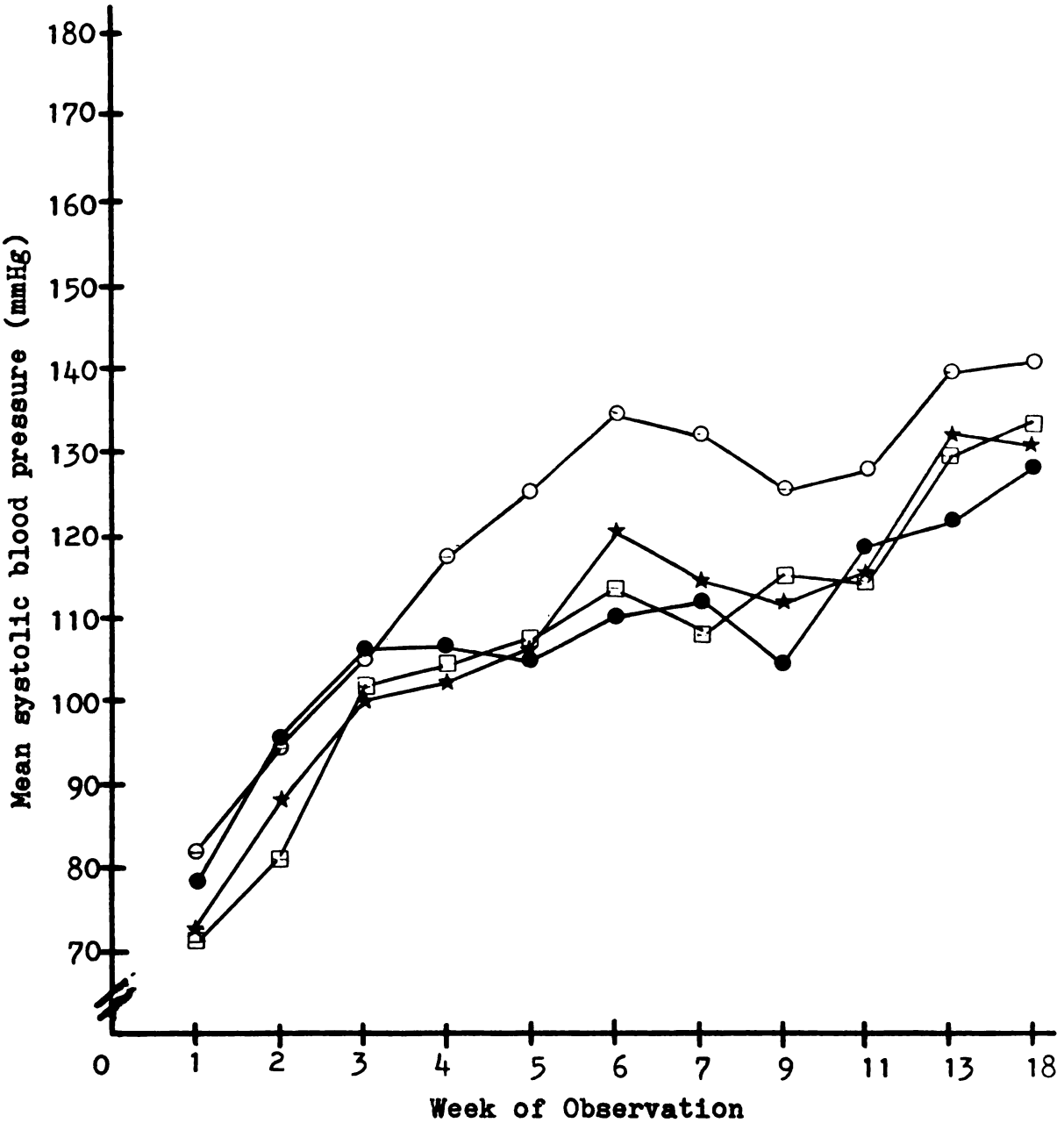

FIGURE 10.--Mean systolic blood pressure of male Osborne Mendel rats fed a grain (●), high fat (★), high sucrose (○), or high cornstarch (□) diet in conjunction with a 1-1.5% NaCl drinking solution for 18 weeks post-weaning.

CONCLUSIONS

Blood pressure was elevated in rats given the hypertonic drinking solutions. As concentration of the solution increased, mean arterial blood pressure increased and became more labile. In rats fed the high fat, high sucrose and high cornstarch diets blood pressure reached hypertensive levels when the rats were given the 2% NaCl drinking solution. Grain-fed rats did not become hypertensive when given the 2% NaCl solution. When given a 1-1.5% NaCl drinking solution only rats fed the high sucrose diet became hypertensive; rats fed the high fat, grain and high cornstarch diets had not reached hypertensive levels at the end of the 18 week feeding period but they were approaching hypertension. When maintained on distilled deionized water blood pressure was highest in rats fed the high sucrose diet, yet all blood pressures remained in the normotensive range.

Pulse rates were highest in rats fed the high sucrose diet regardless of which drinking solution was consumed. The presence of either hypertonic drinking solution did not significantly alter pulse rate over time regardless of the type of diet consumed. However, rats given the 2% NaCl solution exhibited a more labile pulse rate with time than did rats given the 1-1.5% solution or distilled deionized water.

Growth rate was significantly depressed in rats given

the 2% NaCl solution. Rate of growth was not depressed in rats given the 1-1.5% NaCl solution. The retarded growth pattern observed in the former group of rats reflected the marked depression in food intake. Growth rate was most severely depressed in rats fed the high sucrose diet and least in rats fed the grain diet.

Relative weight of the heart, right kidney and right adrenal gland increased in rats given the 2% NaCl solution. Rats fed the high sucrose diet exhibited the largest increase in relative weight of each organ with exception of the adrenal gland which was hypertrophied to the greatest degree in rats fed the high fat diet in conjunction with the 2% NaCl drinking solution.

Concentration of sodium and potassium remained unchanged in the serum and heart of rats given the 2% NaCl solution when compared to rats given the distilled deionized water. Sodium concentration in the kidney and right hind femur reflected total sodium intake and consequently increased in rats given the 2% NaCl solution. Potassium concentration remained unaltered in the kidney of rats given the 2% NaCl solution. In contrast, concentration of potassium in the femur was decreased in these rats.

SUGGESTIONS FOR FURTHER STUDY

Sodium Chloride Absorption

When compared to rats fed the high fat, high sucrose and high cornstarch diets and consuming the 2% NaCl drinking solution, animals fed the grain diet had the lowest mortality and retained the smallest amounts of sodium in tissues of the body. Blood pressure of these rats was not considered to be in the hypertensive range despite the fact that these rats received as much sodium chloride as rats fed the semi-purified diets. It is well established that a 2% NaCl drinking solution produces hypertension in rats (Sapirstien et al. 1950). This raises the question of whether sodium was absorbed by animals fed the grain ration. Further study might include 24 hour feces and urine analysis for sodium and potassium concentrations. This procedure would shed some light on the pathway of sodium once it enters the body of the animal. Although the amounts of fiber and protein in the diets were nearly equal, the type of fiber or protein may have some influence on the absorption of sodium and therefore warrants consideration for investigation as well.

Concentration of Sodium in Kidney and Bone

Concentration of sodium in kidney and bone increased in rats given the 2% NaCl solution. It appears that the concentration of sodium in the kidney and bone reflects total sodium intake. Further study may involve administration of salt solutions of various concentrations to rats and measurement of sodium and potassium in kidney and bone of these animals. The effect of a follow-up period of salt restriction would verify whether the bone was actually a labile source of sodium. Consideration might be given to the effect of a high concentration of sodium in the bone on red blood cell formation. Simple procedures may include blood hematocrits and hemoglobins.

Fructose and Blood Pressure

Since the high sucrose diet appeared most detrimental to health of the rats, some light may be shed on the reason through the addition of a high fructose diet to the experimental design. Fructose has been implicated as the culprit in the sucrose effect on blood pressure (Yudkin 1973) but substantial evidence in support of this fact has not been produced.

Appetite Depression

Miller and Czajka (1967) have decreased gastric emptying time in rats force-fed hypertonic glucose solutions.

The osmotic effect of hypertonic diets in addition to a hypertonic drinking solution may be completely different. Pursuit of gastric emptying time in rats given the hypertonic sodium chloride solutions and fed the various diets is worth consideration. Appetite was definitely depressed in rats given the 2% NaCl drinking solution yet the reason is obscure.

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APPENDICES

APPENDIX A

OPERATION OF ESG COUPLER FOR MEASUREMENT OF BLOOD PRESSURE AND PULSE RATE

1. POWER switch ON
2. RECORD switch OFF
3. VARIABLE control set at 2 OmV/cm
4. POLARITY to positive (+)
5. FILTER switch to 10 Hz position
6. MV/CM switch to 100 mV/cm position
7. Adjust baseline with POSITION control
8. Set ESG coupler PRESSURE range switch to 0-100 mmHg (maximum sensitivity of 20 mmHg/cm with full-scale deflection of 5 cm)
9. RECORD switch ON

APPENDIX B

PROCEDURE FOR CALCULATING TOTAL SODIUM INTAKE

Since the atomic weight of sodium (Na) is 23 and the atomic weight of chlorine (Cl) is 35.5, it follows that the atomic weight of sodium chloride (NaCl) is 58.5.

Each gram of NaCl consumed contains 23/58.5 or 39.3% sodium (Na).

To obtain total sodium intake from fluid intake the total quantity of fluid consumed (ml) was multiplied by the concentration of the NaCl solution. This number was then multiplied by 0.393 to obtain grams of sodium.

Example:

6000 ml fluid x 0.02 x 0.393 = 47.16 gms Na

Since the grain, high sucrose and high cornstarch diets contained 0.5% sodium (Na) and the high fat diet contained 0.75% sodium, total sodium intake from the diet was calculated by multiplying total grams of diet consumed by either 0.005 or 0.0075 depending on the diet in question.

Example:

1400 gm grain diet x 0.005 = 7.0 gm Na

Total sodium intake was the sum of the sodium ingested from both the diet and drinking solution.

47.16 gm + 7.0 gm = 54.16 total gm Na