Effect of Obesity in the Rat on Renal Transport of Organic Acids and Bases[1] (37004)

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Abnormality of kidney function has been associated with obesity in humans by several workers (1–3). An association of kidney damage with increased body weight was reported in hypothalamically obese rats (4–6), in rats with unrestricted feed intake (7, 8), and in genetically obese rats (9). Preliminary experiments in this laboratory suggested that several renal functions (excretion of a phenolsulfonphthalein (PSP) load *in vivo*, urinary concentrating ability, *etc.*) in the dietary obese rat were markedly depressed. Since function tests in the intact animal may be influenced by changes in blood pressure, blood volume, *etc.*, it is difficult to relate abnormalities of renal function tests to specific changes in the kidney. It was, therefore, of interest to utilize an *in vitro* method to assess renal function in tissue from obese animals.

Renal transport of organic acids and bases is an important homeostatic mechanism as it is a means of excretion of potentially toxic by-products of metabolism (10). Active transport of these materials can be quantitated *in vitro* using the slice technique of Cross and Taggart (11). In this technique very thin renal cortical slices are incubated in an oxygenated buffered salt medium and the intracellular accumulation of compounds such as *p*-aminohippurate (PAH) or PSP is used as an indication of the activity and capacity of the secretory system. When the uptake or accumulation of a representative acid and a representative base are measured simultaneously, specific changes in either acid or base transport can be measured, whereas a more generalized depression of proximal tubular function will be reflected as depression of accumulation of both acid and base (12). Thus, this technique seemed to be well suited for the measurement of the changes in renal function in obese animals.

p-Aminohippurate (PAH) was used as the prototype to study organic anion transport while *N*-methylnicotinamide (NMN) was used as the prototype for studying organic cation transport. The experimental model for investigating the physiological effects of obesity was the Osborne Mendel rat fed the high-fat diet described previously (13, 14).

Methods. Osborne Mendel male rats of NIH stock bred in the human nutrition laboratory were fed either a grain ration (GR) (15) or a 60% fat ration (HF) (13) which has been shown to produce obesity in this strain (14). Animals fed the HF or GR diet from weaning up to 75 wk of age, weighing from 150 to 950 g were used in this study. Rats fed the high-fat diet became grossly obese as determined by increased body weight and total body fat content. The animals were housed in a temperature controlled room with a 12–12 hr light–dark cycle. Food and tap water were available *ad libitum.*

Animals were weighed and killed by cervical dislocation or by decapitation. The kidneys were rapidly removed, weighed and placed in iced normal saline. Renal cortical slices of 0.3–0.4 mm thickness were prepared free hand. Approximately 100 mg of tissue was added to beakers containing 2.7 ml of incubation medium. The latter was the phosphate buffer medium devised by Cross and Taggart (11) containing 7.4×10^{-5} *M* PAH and 6.0×10^{-6} *M* NMN-^{14}C (4.6 mCi/mmole) adjusted to pH 7.4.

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Slices were incubated in a Dubnoff metabolic shaker at 25° under a gas phase of 100% oxygen for 90 min. After incubation, slices were quickly removed from the medium, blotted, weighed and placed in a graduated cylinder containing 3 ml of 10% trichloroacetic acid (TCA). A 2 ml aliquot of the medium was taken from each beaker and treated similarly. The tissue was macerated with a glass stirring rod. Slice and medium samples were brought to a final volume of 10 ml with distilled water and centrifuged at 1400 rpm for 10 min. After centrifugation, PAH was determined by the spectrophotometric method of Smith *et al.* (16). When NMN-^{14}C was used, 1 ml of slice or medium supernatant was added to a scintillation vial containing 10 ml of modified Bray's solution (6 g of 2,5-diphenyloxazole and 100 g of naphthalene/liter of dioxane). Radioactivity was measured using a Beckman LS-100 liquid scintillation counter employing external standardization. All samples were counted to an accuracy of ± 2.00%.

Transport was expressed as the slice to medium (S/M) ratio which represents the concentration of PAH per gram of tissue (wet weight) divided by the concentration of PAH per milliliter of medium or, in case of NMN-^{14}C, disintegrations per minute per gram of tissue (wet weight) divided by the disintegrations per minute per milliliter of medium. Data were analyzed statistically using Student's t test and regression analysis (17). In all statistical tests, the 0.05 level of probability was used as the criterion of significance.

Results. The ability of renal cortical slices to accumulate PAH was inversely correlated with age, body weight and kidney weight (Fig. 1). As any of these variables increased, the transport of PAH decreased. The inverse relationships were significant with GR, HF or all data pooled ($p < 0.05$).

Organic acid accumulation by renal cortical slices was significantly depressed in HF animals. The depression was most pronounced in the younger animals (Fig. 1). When the data from all ages were pooled (Fig. 2), the PAH S/M ratio in slices from HF animals, 4.81 ± 0.29 (mean ± SE) was significantly less than the 6.96 ± 0.41 developed in slices from GR animals.

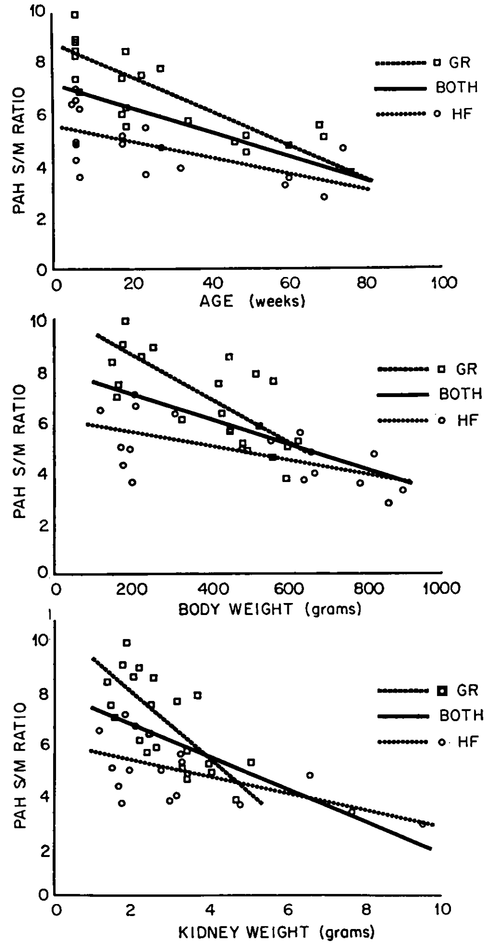

FIG. 1. Accumulation of PAH (S/M ratio) in renal cortical slices from GR (□) and HF (○) male rats plotted against age (top), body weight (center) and kidney weight (bottom). The calculated regression lines for GR (- - -), HF (• • •) and all animals independent of diet (—) are plotted. Points are the average of duplicate determinations for individual rats. All lines demonstrate significant regression ($p < .05$).

Accumulation of NMN by renal cortical slices was significantly correlated with age and kidney weight in the HF group but not in the GR group (Fig. 3). NMN accumulation was not correlated with body weight in either group (Fig. 3).

The accumulation of NMN by renal cortical slices from HF animals was no different from that of GR animals (Fig. 4). Slices

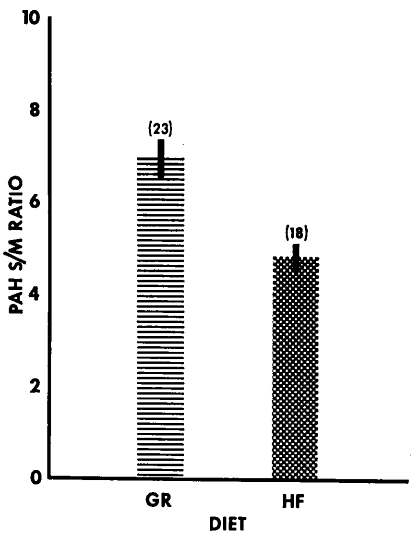

FIG. 2. Accumulation of PAH (S/M ratio) in renal cortical slices from male rats of different ages fed the control grain ration (GR) or the experimental high-fat ration (HF). Each bar represents the mean ± (SE) obtained from duplicate determinations on the number of animals shown in parentheses. The value obtained from the HF group is significantly less than that of the GR group ($p < 0.05$).

from animals fed GR incubated under the conditions as described previously (90 min, Cross and Taggart medium, 100% oxygen) developed NMN S/M ratios of 6.75 ± 0.33 (Fig. 4). This was not significantly different from the NMN S/M ratios (6.55 ± 0.40) obtained when slices from HF animals were incubated similarly.

Discussion. Organic acid transport is a specific function of the renal proximal tubule. This is in contrast to more generalized functions such as sodium reabsorption.

Because of the large load delivered to the tubule for reabsorption, sodium transport utilizes the bulk of the energy expenditure of the kidney. Only a small fraction of this energy expenditure is necessary for such functions as PAH transport. Normally the sodium reabsorptive mechanism is not operating at full capacity and is not challenged by the filtered sodium load. Thus a small decrement in energy availability might not affect overall sodium transport. In the present experiments it was possible to challenge the organic transport system by measuring uptake in the steady state. It was reasoned that when so challenged subtle changes in this function might be observed prior to the development of nephropathies in the intact animal. This assumption appears to be correct for a significant correlation in PAH transport was seen with age, body weight and kidney weight with both diets (Fig. 1).

Renal tubular transport of PAH estimated as accumulation of this compound by renal

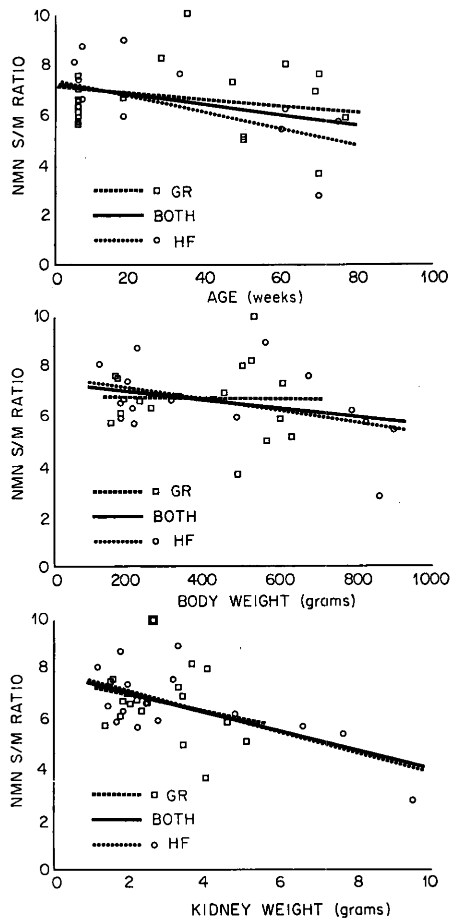

FIG. 3. Accumulation of NMN (S/M ratio) in renal cortical slices from GR (□) and HF (O) rats plotted against age (top), body weight (center) and kidney weight (bottom). The calculated regression lines for GR (- - -), HF (• • •) and all animals independent of diet (—) are plotted. Points are the average of duplicate determinations for individual rats. NMN accumulation in HF animals is correlated with age and kidney weight.

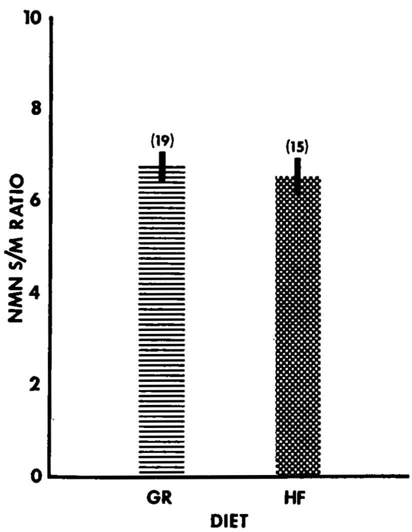

FIG. 4. Accumulation of NMN (S/M ratio) in renal cortical slices from male rats of different ages fed GR and HF. Each bar represents the mean ± (SE) obtained with duplicate determinations on the number of animals in parentheses. The values obtained are not significantly different ($p > 0.05$).

cortical slices (S/M ratio) was significantly depressed in obese animals compared to controls (Fig. 1). Depression of PAH accumulation by renal cortical slices with age confirms work of Adams and Barrows (18) and Beauchene, Fanestil and Barrows (19).

The specificity of the depression of transport of organic acids in the HF group was determined using NMN as a prototype to study base transport. Accumulation of PAH by renal cortical slices in the Cross and Taggart incubation system may be specifically influenced by a number of factors (*i.e.*, substrate stimulation, inhibitors, *etc.*) without any change in NMN transport (12, 20–21). As with PAH, NMN transport was inversely related to age and kidney weight in the HF group (Fig. 3). Body weight, however, was not significantly related to base transport (Fig. 3). Accumulation of NMN by renal cortical slices from animals fed the GR and HF was not different (Fig. 4). Thus, the effect of obesity on transport appears to be specific for organic acids.

Summary. The effect of obesity on accumulation of PAH by rat renal cortical slices was determined using an *in vitro* slice technique. Accumulation of PAH was significantly depressed in animals made obese by feeding a high-fat diet. Accumulation of PAH decreased with increasing age, body weight and kidney weight independent of diet.

Organic base accumulation was determined to demonstrate the specificity of the effect of the high-fat diet and obesity on organic acid transport. Accumulation of the base NMN was not different in renal tissue from grain-fed and high-fat-fed animals. There was no correlation between NMN accumulation and body weight. In high-fat-fed animals NMN accumulation by renal cortical slices decreased with increased age and kidney weight. Age and kidney weight in the grain-fed animals was not related to NMN accumulation.

The data suggest that obesity and/or the constituents of the high-fat diet have a depressant effect upon the kidney. Since NMN accumulation was not influenced it is concluded that the effect is not a generalized depression of renal function but selective depression of organic anion transport.

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