Factors Contributing to Depressed Renal Transport of Organic Anions in the Obese Rat[1] (37509)

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Obesity in humans is often accompanied by abnormalities in renal function (1–3). Most experimental models of obesity similarly display nephropathies (4–9). An association of kidney damage with excessive body weight was reported in hypothalamically obese rats (4–6), in rats with unrestricted feed intake (7–8), and in genetically obese rats (9).

Previously we reported that renal transport of the organic acid, *p*-aminohippurate (PAH) was significantly depressed in male rats made obese by feeding a 60% fat diet (10). In contrast, the transport of the organic base, *N*-methylnicotinamide (NMN), was unaffected by the obesity. Inasmuch as depression of PAH transport occurred in tissue prior to any effect on NMN transport, the data suggested that the depression was a specific biochemical lesion and did not reflect generalized depression of kidney function in obesity. The experiments reported here were designed to elucidate factors responsible for the depression of PAH transport in obese animals.

Materials and Methods. The Cross and Taggart (11) slice technique was used to quantitate the ability of renal cortical slices to actively accumulate organic anions. PAH was used as the prototype to study organic anion transport while NMN was used as the prototype for studying organic cation transport.

Osborne Mendel male rats of NIH stock bred in the Human Nutrition Laboratory were fed from weaning either a control grain (GR) ration (12) or a 60% fat (HF) ration (13) which has been shown to produce obesity in this strain (14). 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, trimmed free of capsule and fat, 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 incubated in the phosphate buffer medium devised by Cross and Taggart (11) routinely 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. In uptake studies the medium contained PAH at concentrations of 2, 4 or 8 $\times 10^{-4}$ *M*. Incubations and determinations of PAH and NMN were carried out as previously described (15).

Slices were incubated for 2 and 12 min in order to estimate the rate of PAH transport. Transport was calculated as the difference in the amount of PAH accumulated per gram of slice per minute between 2 and 12 min incubation. A Lineweaver-Burk plot (16) was used to express the results.

PAH accumulation during a 90 min incubation period was expressed as the slice/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 media or in the case of NMN-^{14}C, disintegrations per minute per gram of tissue (wet weight) divided by the disintegrations per minute per milliliter of medium.

To determine renal tissue lipid and moisture composition, slices were prepared as de-

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TABLE I. Kinetic Analysis of PAH Uptake in Rat Renal Cortical Slices.[a]

Diet[b]	Age (wk)	Slope[c]	K_m (10^{-4} moles/liter)[c]	V_{max} (μg/g/min)[c]
GR	12	0.391	15.62	39.37
HF	12	0.360	5.37	14.92
GR	60	0.490	6.10	12.45
HF	60	0.318	1.47	4.63

[a] Rate of PAH uptake (μg PAH/g kidney slice/min) at PAH concentrations of 2, 4 and 8×10^{-4} *M* was determined by measuring the difference in accumulation after 2 and 12 min of incubation. Triplicate determinations of uptake for each diet and age were made. Kidneys from 3 animals were pooled for 1 determination.

[b] Diets used were grain ration (GR) and high fat (HF).

[c] Values were obtained from the Lineweaver-Burk plot shown in Figs. 1 and 2.

scribed previously and stored in iced saline until blotted, weighed and dried in an oven for 24 hr at 100°. Tissue water content of cortical slices was determined as the difference between the wet and dry weights, expressed as percentage of the wet weight. After drying, the total ether-extractable fat content of the renal cortical slices was determined in the dried sample using the Goldfisch apparatus. In some cases, it was necessary to pool dried tissue samples for fat determinations. The content of fat was expressed on a wet or dry basis as percentage of wet or dry tissue weight.

Oxygen consumption of slices was determined using a multiple-unit constant pressure microrespirometer (17). Oxygen consumption was calculated on wet weight (w) and dry weight (d) bases. QO_{2_w} and QO_{2_d}, were used to express the microliters of O_2 per mg wet or dry tissue per hour, respectively. All values were corrected to standard temperature and pressure (STP).

A diet switching technique was used to determine the effect of the diet *per se* on PAH and NMN accumulation. Animals fed GR or HF from 1 to 45 wk were switched to the other diet for 2 days to 3 wk. PAH and NMN accumulation within the same age rats were unaffected by the length of time the first and second diets were fed; therefore, all values within the same dietary treatment were pooled for analysis.

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

Slices for histological study from obese and control animals were placed in Zenker's solution (18) (50 g potassium dichromate, 70 g mercuric chloride, and water to 2000 ml) for 10–12 hr, washed overnight in cold running tap water to remove excess mercury salts and placed in 70% ethanol in coded vials. A double-blind study was made to determine if histological differences between renal cortical slices from GR and HF-fed rats could be demonstrated.[2] Kidneys were imbedded in paraffin and sections 2 μm thick prepared. Sections were stained with hemotoxylin and eosin and periodic acid–Schiff's (PAS) (18, 19).

Data were analyzed statistically using Student's *t* test, group comparison (20). In all statistical tests, the 0.05 level of probability was used as the criterion of significance.

Results. The maximal rate of PAH uptake by renal cortical slices from 12-wk-old GR

[2] The valuable assistance of Joan Mattson, M.D., Department of Pathology, in evaluating the histological sections is appreciated.

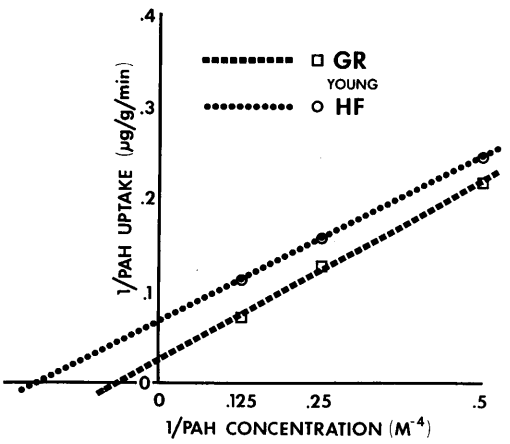

FIG. 1. Kinetic analysis of PAH uptake by renal cortical slices from 12-wk-old rats fed GR (□) or HF (○) using the Lineweaver-Burk plot. The rate of PAH uptake (μg/g/min) at PAH concentrations of 2, 4 and 8 × 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 min. The points indicate the means from 3 experiments. The slopes of the curves are 0.360 (HF) and 0.391 (GR). Calculated values for K_m (× 10^{-4} moles/liter) are 15.62 (GR) and 5.37 (HF) and for V_{max} (g/g/min) are 39.37 (GR) and 14.92 (HF).

rats was greater than that by slices from HF rats of the same age (Fig. 1). The slopes of the lines for GR and HF animals were 0.391 and 0.360, respectively (Table I). The plot for the GR group exhibited a V_{max} of 39.37 (μg/g/min) while the V_{max} for the HF group was 14.92 (Table I). K_m values for GR and HF animals were 15.62 and 5.37 (× 10^{-4} moles/liter), respectively (Table I).

The rate of PAH uptake by renal cortical slices from 60-wk-old GR rats was greater than that for slices from HF rats of the same age (Fig. 2). The slopes of the lines for GR and HF animals were 0.490 and 0.318, respectively. The plot for the GR group exhibited a V_{max} of 12.45 (μg/g/min) while the V_{max} for the HF group was 4.63 (Table I). K_m values for GR and HF animals were 6.10 and 1.47 (× 10^{-4} moles/liter), respectively (Table I).

There were no significant differences in water or total ether-extractable fat content of the slices from the GR or HF groups (Table II). Oxygen consumption by renal cortical slices from kidneys of rats fed GR and HF were not significantly different on a dry or a wet weight basis (Table III).

Histological examination of the kidneys revealed both groups were normal for rats of the age used (30 wk old) (Fig. 3). There were no apparent morphological differences in kidney sections from HF and GR animals examined by light microscopy (Fig. 3). In both groups tubules were normal except for slight dilation. Infrequent casts were present in kidneys from both groups. No evidence of

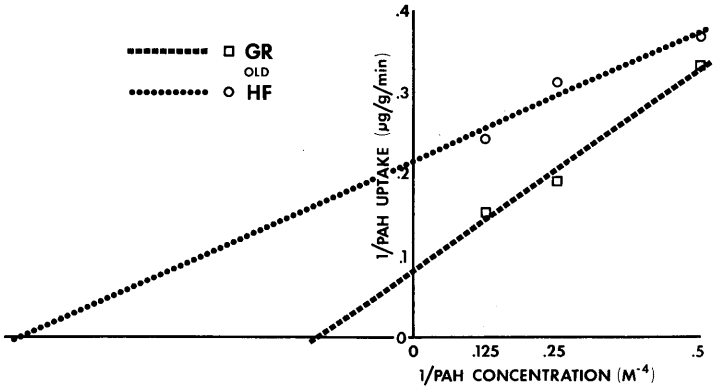

FIG. 2. Kinetic analysis of PAH uptake by renal cortical slices from 60-wk-old rats fed GR (□) or HF (○) using a Lineweaver-Burk plot. The rate of PAH uptake (μg/g/min) at PAH concentrations of 2, 4 and 8 × 10^{-4} M was determined by measuring the difference in accumulation after 2 and 12 min. The points indicate the means from 3 experiments. The lines are calculated regression lines. The slopes of the curves are 0.490 (GR) and 0.318 (HF). Calculated values for K_m are (× 10^{-4} moles/liter) are 6.10 (GR) and 1.47 (HF) and for V_{max} (g/g/min) are 12.45 (GR) and 4.63 (HF).

TABLE II. Approximate Composition of Renal Cortical Slices from Rats Fed the Grain Ration or High Fat Ration.

Diet	No.	% Moisture	Ether-extractable % fat (dry wt basis)
GR[a]	9	86.56 ± 0.69	8.28 ± 0.60
HF	7	88.46 ± 1.32	9.85 ± 2.33

[a] Diets used were the grain ration (GR) and the high fat ration (HF).

kidney infection or inflammation was seen. Glomerular changes observed in kidneys from both dietary groups included focal hypercellularity, increased mesangial tissue, and limited metaplasia.

Since previous results (10) suggested that accumulation of PAH by renal cortical slices was depressed in animals fed HF compared to GR, the effect of the diet *per se* was investigated. Animals fed HF for any period of time had depressed accumulation of PAH when compared to animals fed GR for a comparable period of time (Table IV). Animals fed GR and switched to HF at least 2 days prior to sacrificing had renal cortical slices which accumulated PAH as if the animals had been on the HF ration throughout the experiment (Table IV). Accumulation of PAH by renal cortical slices from HF animals was significantly less than that by slices from GR animals.

Animals were fed HF and switched to GR at least 2 days prior to being sacrificed. Kidney slices from these animals developed PAH *S/M* ratios not significantly different from those obtained with kidneys from animals fed GR continuously (Table IV). Accumulation of PAH by kidney slices from animals fed GR or HF and switched to GR was significantly greater than that by kidney cortical slices from animals fed HF or GR and switched to HF (Table IV).

NMN accumulation by renal cortical slices was unaffected by diet switching (Table IV). When animals were fed GR and switched to HF, accumulation of NMN was not significantly different from that by renal cortical slices from animals fed HF and switched to GR (Table IV). This was true when the initial ration was fed for from 1 to 45 wk and the second ration for 2 to 21 days.

Since these results indicated that the depressed accumulation of PAH by renal cortical slices was due in large part to the diet, the effect of serum on accumulation was determined. Kidney slices from animals fed GR or HF were incubated with the normal media plus serum from GR or HF animals (Table

TABLE IV. Effect of Diet on Accumulation (*S/M* Ratio) of PAH and NMN by Slices of Rat Renal Cortex.[a]

Diet[b]	PAH *S/M* ratio[d]	NMN *S/M* ratio[d]
GR	6.96 ± 0.41 (23)[c]	6.75 ± 0.33 (19)[c]
HF	4.81 ± 0.29[e] (18)	6.55 ± 0.40 (15)
GR→HF	4.63 ± 0.30[e] (9)	7.03 ± 0.34 (7)
HF→GR	6.48 ± 0.73 (5)	8.36 ± 0.65 (3)

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

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

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

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

[e] Significantly different from GR ($p < 0.05$).

TABLE III. Oxygen Consumption of Renal Cortical Slices from Rats Fed the Grain Ration or High Fat Ration.

Diet	No.	μl/hr/mg tissue	
		Wet	Dry
GR[a]	4	2.20 ± 0.24	12.84 ± 0.99
HF	4	2.52 ± 0.09	11.97 ± 1.32

[a] Diets used were grain ration (GR) and high fat ration (HF).

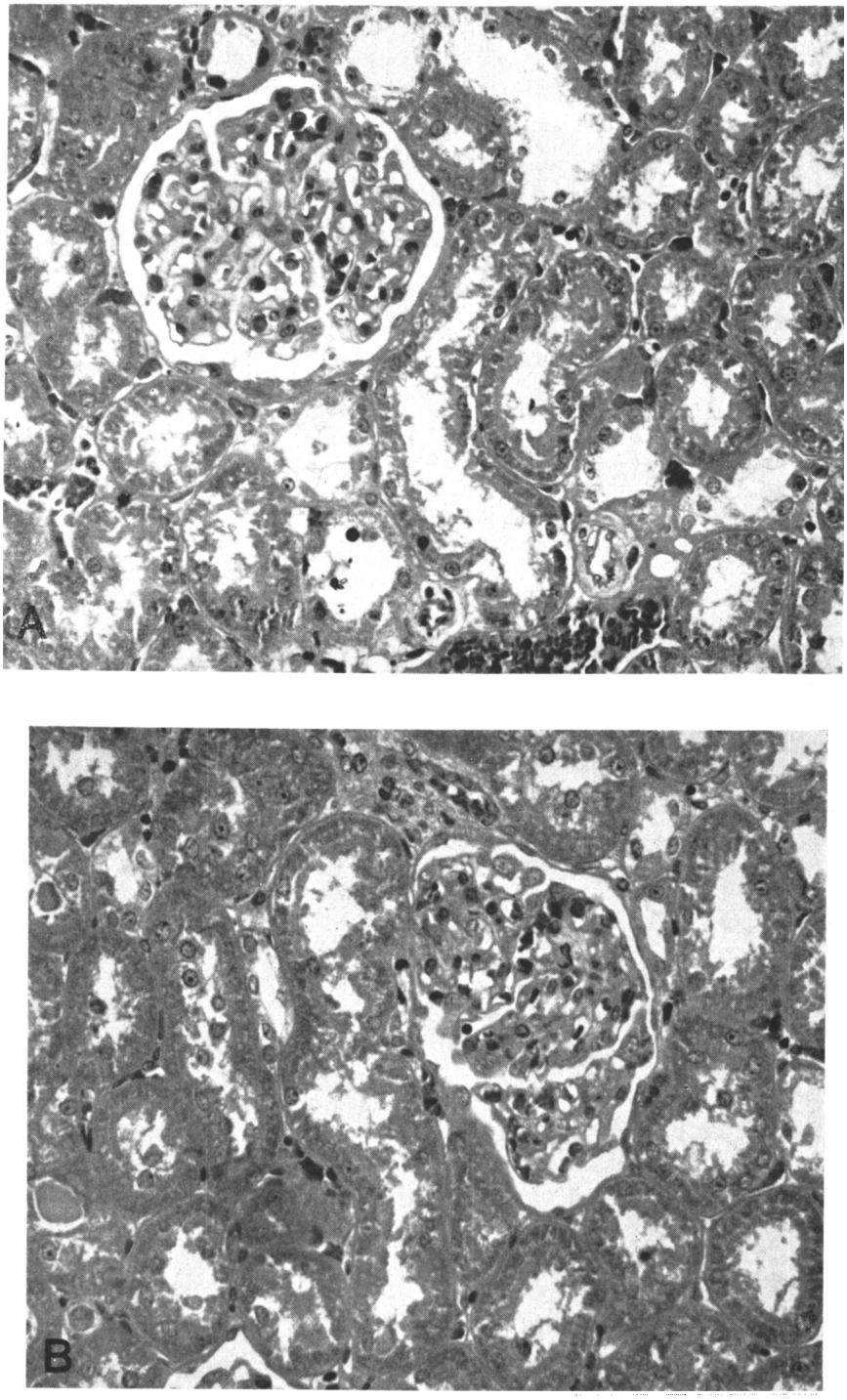

FIG. 3. Kidney sections from a typical HF (A) and GR (B) animal 30 wk of age. Tubules are slightly dilated and vacuolated with infrequent casts. Glomeruli have normal basement membranes; Bowman's spaces are empty. Occasional glomeruli show metaplasia. Severe congestion is probably an artifact. Measured PAH S/M ratio: A = 3.75; B = 8.51.

TABLE V. Accumulation of PAH (*S/M* Ratio)[a] by Slices of Rat Renal Cortex in the Presence of Serum.

Incubation medium[b]	Serum source	Kidney source	No.[d]	PAH *S/M* ratio
2.5 ml medium + 0.5 ml serum	HF[c]	GR[c]	5	15.38 ± 0.21[e]
	HF	HF	4	13.45 ± 1.27[e]
	GR	GR	3	14.73 ± 0.02[e]
	GR	HF	3	15.08 ± 1.65[e]
2.5 ml medium + 0.5 ml saline	—	HF	4	4.25 ± 0.02[e]
	—	GR	4	7.28 ± 0.25
2.7 ml medium	—	HF	18	4.81 ± 0.29[e]
	—	GR	23	6.96 ± 0.41

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

[b] Cross and Taggart (11) medium with PAH concentration of 7.4×10^{-5} *M* was used.

[c] Diets used were grain ration (GR) and high fat ration (HF).

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

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

V). Accumulation of PAH by renal cortical slices incubated with medium plus saline was used as a control against dilution effects. In all cases, accumulation of PAH by renal cortical slices was enhanced by serum independent of the source of serum or kidneys (Table V). Accumulation was unaffected by addition of an equal volume of saline (Table V). PAH *S/M* ratios developed by renal cortical slices from HF and GR animals incubated in HF serum were not different. Those developed by renal cortical slices from HF or GR animals with the addition of GR serum were not different. Furthermore, there were no differences in PAH *S/M* ratios developed when HF serum was used compared with GR serum.

The *S/M* ratios obtained with all serum–kidney combinations were significantly greater than those with the dilution control (2.5 ml medium + 0.5 ml saline) or with the control medium (2.7 ml). There were no significant differences in the PAH *S/M* ratios obtained in 2.5 ml medium plus 0.5 ml saline when compared with 2.7 ml medium (Table V).

Discussion. Parallel systems for transport of organic acids and bases are located in the renal proximal tubules. Selleck and Cohen (21) suggested that the primary function of the organic acid transport system is to move specific products of intermediary metabolism (nonesterified fatty acids, α-ketoglutarate, citrate, *etc.*) to sites of dissimilation in the kidney and liver. The importance of the organic base transport system is less apparent, although a number of naturally occurring plasma constituents (thiamine, choline, NMN, guanidine, piperdine and methyl guanidine) are secreted into the urine by this system (22).

The *in vitro* slice technique is a reliable estimate of organic acid and base transport *in vivo*. In the rabbit the maximum capacity of the renal cortex to concentrate PAH *in vivo* is 4–5 μmoles/g tissue, and similar values have been obtained using kidney slices (23). Penicillin, PAH, or carinamide, competitive inhibitors of the acid transport system, have similar action, both *in vivo* and *in vitro*, on the intracellular accumulation of phenol red (24). Other inhibitors such as dibenamine and dibenzyline depress NMN transport *in vitro* and *in vivo* (25). Mudge and Taggart (26) demonstrated that acetate and lactate have stimulatory effects on PAH transport in both preparations while succinate and fumarate uniformly depressed transport.

The *S/M* ratio reported in this study was measured in a steady state system and thus represents binding and runout capacity of the renal tissue as well as transport capacity.

Therefore, the S/M ratio is a measure of the ability of the tissue to maintain a concentration gradient. Depressed PAH S/M in HF animals could result from an alteration in entry, accumulation and/or runout of PAH, in nonspecific binding of PAH to protein and in extracellular water, intracellular water or protein content of the renal cortical slice.

During short periods of incubation (as between 2–12 min) uptake of PAH is linear, suggesting that intracellular accumulation of the compound is not sufficiently high to alter the rate of influx. Thus the uptake over short periods of time indicates the rate of transport of a material into the tissue (27). The renal transport system as studied is not a pure system and renal organic acid transport probably does not follow true Michaeles-Menten kinetics. Nevertheless, the Lineweaver-Burk plot remains one of the most useful tools available to study these transport kinetics (28–30). In the Lineweaver-Burk plot, the x-intercept ($1/K_m$) could reflect the relative affinity of a carrier substance for PAH and the y-intercept ($1/V_{max}$), the maximal velocity of the PAH accumulation process. On the basis of these assumptions the data presented in Figs. 1 and 2 suggest that the apparent affinity and maximal velocity are both different when values for GR animals are compared with HF animals within the same age group. These changes in kinetics suggest that PAH transport was inhibited in a noncompetitive manner in the HF animals. Another likely interpretation is that the inhibitor–carrier interaction is nonreversible or slowly reversible and that Michaelis-Menten kinetics are not applicable. Apparently the inhibitor was not released even after the renal cortical slices remained in saline or in the Cross and Taggart medium for 90 min. The presence of such an inhibitor is consistent with the observation that differences in accumulation of PAH by renal cortical slices from HF and GR rats was not reflected in changes in slice composition, (Table II), oxygen consumption (Table III) or histology (Fig. 3). In the intact animal, an inhibitor could accumulate and eventually impede the transport system. Such a possibility was suggested by Balagura-Baruch and Stone (31). These workers reported that α-ketoglutarate inhibited PAH secretion in dogs by a noncompetitive or mixed mechanism.

Since the high fat diet fed to produce the obesity and the control grain ration differed with respect to caloric density, and source of protein, fat, and carbohydrate, the effect of the diet *per se* on the accumulation of PAH by rat renal cortical slices was determined using a diet switching technique. When diets were switched, accumulation of PAH by renal cortical slices from animals fed HF for any period of time and switched to GR for any period of time just prior to sacrifice (Table IV) was similar to that by kidneys from animals fed GR throughout (Table IV). Animals fed GR and switched to HF for any period of time had kidneys in which transport of PAH was significantly less than that for animals fed GR prior to sacrifice. Thus diet *per se* appeared to be a primary factor influencing the final S/M ratio, although age, body weight and kidney weight influenced the accumulation of PAH by renal cortical slices (10). The effect of diet on the accumulation of PAH was easily reversible and the time required for this change was no longer than 2 days. NMN accumulation was unaffected by diet or diet switching (Table IV).

Since the effect of diet appeared readily reversible, it was reasoned that a dietary metabolite in the serum might be directly affecting accumulation of PAH in the *in vitro* system. However, when serum was incubated with the kidney slices, PAH accumulation was enhanced. Serum from HF animals stimulated the accumulation of PAH by kidney slices from both GR and HF animals (Table V). Similarly, GR serum added to the incubation medium significantly increased the accumulation of PAH by kidney slices from both groups (Table V). These results confirm the effect of normal serum in enhancing PAH accumulation by renal cortical slices reported by Orringer, Weiss and Preuss (30). If some factor of dietary origin in the serum of the HF animals depressed PAH accumulation by renal cortical slices, its concentration was insufficient to produce an inhibitory effect when added to the incubation system. Apparently, the factor(s) responsible for the enhancement

of PAH accumulation is in sufficient concentration to overcome any inhibitor that might be present in the HF serum. This is in contrast to reports that uremic serum (32, 33) or serum from nephrectomized animals (15), when added to this incubation system, depressed accumulation of PAH by renal cortical slices.

Weiner and Mudge (34) proposed that secretion of exogenous compounds by the kidney is always observed under conditions of at least partial competitive inhibition by endogenous compounds. Schachter and coworkers (35, 36) suggested that enhancement of PAH secretion by acetate resulted from removal of these inhibitors from the transport system. Apparently addition of serum to renal cortical slices produces a similar removal of inhibitors. The depressed PAH accumulation of kidney slices from HF rats could be increased to a level equal to that of slices from GR rats by the addition of the serum. Thus, there is no difference in the inherent functional capacities of the kidneys from the obese and control rats for PAH transport. It is concluded, therefore, that in obesity significant changes in kidney function measured *in vivo* and *in vitro* may be a consequence of the dietary constituents and do not necessarily represent obligatory relationships with the obese state.

Summary. The effect of obesity produced by feeding rats a 60% fat diet (HF) on accumulation of the organic anion *p*-aminohippurate (PAH) by renal cortical slices was determined using an *in vitro* technique. The rate of PAH uptake was determined and analyzed kinetically using a Lineweaver-Burk plot. In control rats fed a grain ration (GR) the V_{max} and K_m were less in old (60 wk) than in young (12 wk) animals. The animals on high fat (HF) had V_{max} and K_m values less than the respective age controls. The decrease in apparent affinity and maximal velocity with age and diet could indicate noncompetitive inhibition. The differences observed in PAH transport in the HF animals were not the result of differences in oxygen consumption, histology (light microscopy) or composition of renal cortical slices. Kidneys from animals fed HF and switched to GR at least 2 days prior to sacrificing accumulated PAH as well as those from animals fed GR from weaning. Kidneys from animals fed GR and switched to HF for at least 2 days had depressed PAH accumulation. Organic cation transport as determined with *N*-methylnicotinamide (NMN) accumulation was unaffected by diet switching. These data indicate the presence of some factor inhibiting PAH accumulation, perhaps of dietary origin (as another organic acid), in the serum. However, stimulation, not inhibition, was demonstrated when serum from the HF or control animals was added to the incubation medium. The data are consistent with the hypothesis that in the HF animals there is some factor which is probably tightly bound in the kidney but is present in such low concentrations in the serum that no acute inhibitory effects on PAH accumulation are demonstrable *in vitro*.

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