TABLE II. Statistical Comparison of Mean Estimated Hepatic Blood Flow of a Group of 22 Splenectomized Dogs with Means of Groups of Anesthetized and Unanesthetized Intact Dogs Previously Reported by One of Us.

	Intact				Splenectomized
Anesthetized	No	Yes	Yes	Yes	Yes
No. animals	11	17	14	14	22
No. observations	11	49	49	41	22
Mean flow, ml/min.	633	690	649	650	615
S. E.	44	52	25	28	48
"t"	.28	1.09	.63	.63	
p*	>.70	>.30	>.50	>.50	
Reference No.	5	6	7	8	

* Based on No. of observations.

cant, and as a potential source of error(3) is less important than those induced by other factors(10).

Summary. Hepatic blood flow of chronically splenectomized dogs estimated by the bromsulphalein method was found not to be significantly different than that found in normal intact anesthetized and unanesthetized dogs.

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Renal Tubular Transport of Some Nitro and Amino Hippuric Acids.* (27132)

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Meta and *para* nitrohippuric acids are poorly transported by the renal tubule as judged by low accumulation of these substances in slices of rabbit renal cortex *in vitro*(1). Such a conclusion is not consistent with our observations with *m*-nitrohippuric acid and *p*-nitrobenzoylsarcosine both of which showed effective excretory transport in the dog kidney(2,3). Further examination of the excretion of these nitro compounds in comparison with the excretion of corresponding amino compounds was made, with particular attention to urinary pH.

Experimental. The acid dissociation constants, as taken from the literature or measured by potentiometric titration, are shown in Table I. The partition coefficients were determined by equilibrating solutions of the compounds in N/10 HCl with tributyrin. The values for the amino compounds are somewhat ambiguous, since some protonation of the amino groups might be expected at this pH, leading to ionization and reduction of

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lipoid solubility. Binding to plasma proteins was measured by ultrafiltration. The value for PAH was derived from Taggart's(4) value of 0.92 by eliminating the correction for water content. The value for *p*-nitrohippuric acid did not vary with concentration of the compound and was the same at pH 7.15 and 7.71. The binding of *m*-nitrohippuric acid varied with its concentration; more data are given in(2).

Urinary excretion of these compounds under varying conditions of urinary pH was studied in 15 dogs in chloralose anesthesia (60-100 mg/kg). The majority of the experiments were of the type shown in Fig. 1 and 2. The compounds were given in a priming dose of 0.3 mmol/kg with subsequent infusion of 7.5 μmol/kg per minute. Under these circumstances a plasma concentration of approximately 1 mM was maintained, and such a concentration appears to be more than adequate to saturate the transport system. Analysis of the experimental compounds was achieved by diazotization and coupling(5) which in the case of the nitro compounds was preceded by Zn-HCl reduction. Reduction of the nitro compounds in the body was shown to be negligible by diazotization and coupling without previous reduction with Zn-HCl. The exogenous creatinine clearance was used to measure the rate of glomerular filtration. Urine for analysis was collected from one ureter; that from the other ureter was led through an electrode assembly for continuous observation of pH. Samples of blood for analysis and measurement of pH were taken from the *arteria genus suprema,* and in some cases also from the renal vein for measurement of pH. Plasma proteins were precipitated with cadmium hydroxide, except with *m*-nitrohippuric acid for which trichloracetic acid was used since it gave better recovery. The fluid for intravenous infusion was given at a rate of 1 ml/kg/minute, and at the start of experiment was KCl 4 mM, $CaCl_2$ 0.5 mM, and NaCl 140 mM. Subsequently NaCl was omitted and $NaHCO_3$ was added in concentrations ranging from 0.15-0.45 M. At the beginning of experiment, mannitol 0.8 g/kg and HCl, an average of 3.3 meq/kg, were given

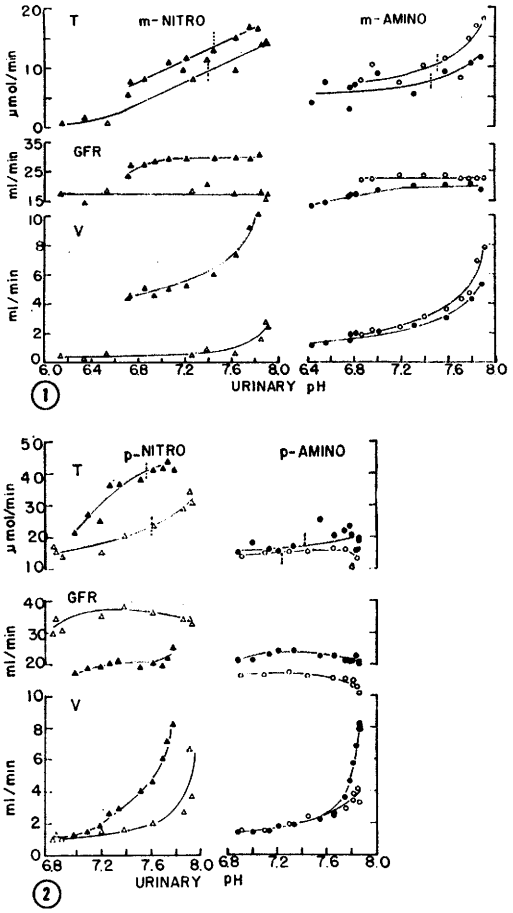

FIG. 1 and 2. Influence of $NaHCO_3$ loading on urinary pH and volume, glomerular filtration rate, and transtubular movement (T) of *meta* (Fig. 1) and *para* (Fig. 2) nitro and amino hippuric acids. Results of 2 different experiments with each compound. Each point represents one 10-min. period of collection of urine. Vertical broken lines indicate point at which the interpolated pH of urine and plasma were the same.

intravenously. The HCl was 0.1-0.5 N in 0.15 M NaCl, given over a 30-minute period to avoid hemolysis. Periods of collection of urine were 10 minutes in duration; blood samples were taken at alternate periods. Transtubular movement (T) was calculated as the difference between excretion and filtration (glomerular filtration rate times plasma concentration times *f*).

Results. Representative experiments are presented in Fig. 1 and 2. Urinary volume rose with the pH on bicarbonate loading. Glomerular filtration rate showed a tendency to increase in some but not all experiments.

TABLE I. Physical Data on Compounds.

Hippuric acid	pK_a	B†	Comparative tendency to reabsorption‡	f§
meta nitro	3.54	2.40	55.4	.64
para "	3.40	2.26	37.7	.84
meta amino	4.18*	.434	47.3	1.00
para "	3.83*	.036	1.74	.98

* From Smith, *et al.*(5).

† Partition coefficient, tributyrin/H_2O.

‡ At pH 7 $\frac{10^6 \cdot B}{M^{1/2}(1 + 10^{7-pK_a})}$ M = molecular wt.

§ f = ratio of concentrations, ultrafiltrate/plasma, at a plasma conc. of 1 mM.

With PAH, calculated tubular excretory transport was independent of pH but increased with the pH in the case of the other compounds.

Discussion. In column 4 of Table I values are given for the calculated comparative rates of passive movement of these substances across cellular membranes applying classical theory of diffusion as found by us to describe the comparative tendency of other compounds to undergo passive reabsorption in the renal tubule(2,6). Such a formulation appears applicable to the findings described here as follows. First, the actual rate of tubular excretory transport is the same at all pH values, but passive reabsorption of the undissociated forms of the nitro and *m*-amino compounds occurs at low pH resulting in a low calculated value for tubular excretion. Secondly, progressive alkalinization, which diminished urinary concentration of the undissociated form, reduces passive reabsorption and calculated tubular excretion increases. Decreasing tubular reabsorption of water also probably contributes to this change.

A possible alternative explanation is that urinary excretion increases as a result of "ion trapping", *i.e.*, passive movement of the undissociated form from interstitial fluid into the lumen. This seems unlikely for 2 reasons. First, urinary excretion increases on $NaHCO_3$ loading while urinary pH is still below that of plasma. Secondly, although urinary pH finally exceeds that of plasma, the difference is seldom more than 0.2 pH unit. Therefore, the tendency of the concentration of the undissociated form in the lumen to decrease as a result of rise in pH is less than the tendency to increase as a result of the tubular reabsorption of water.

That tubular excretion of PAH was independent of urinary pH supports the concept that PAH has little tendency to reabsorption. Hence, its calculated rate of tubular excretion at all the observed pH values appears close to the real rate of tubular excretory transport. On the other hand, if the above premises are accepted, an increase in calculated tubular excretion of the other compounds as urinary pH is elevated suggests that the values obtained at the highest pH are closer to the real rate of tubular excretory transport.

Since tubular excretory rate of the nitro compounds was not less than that of the corresponding amino compounds, the present results are at variance with conclusions drawn from experiments on renal cortical slices *in vitro*(1). In the slice experiments, passive movement of readily diffusible substances may conceivably prevent accumulation. If so, a low slice to medium ratio need not necessarily reflect poor transport. A similar conclusion was reached from our studies of some acetamidobenzoates(6).

Summary. Renal disposal of *m* and *p* nitro and amino hippuric acids was examined in the dog. Administration of sodium bicarbonate did not change urinary excretion of PAH, but increased that of the other compounds. The latter may undergo tubular reabsorption, in accord with their greater lipoid solubility. The 4 substances do not differ in their maximal rates of tubular excretory transport.

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