clopropane and 10 each received ether and pentobarbital sodium as the pre-hypothermic anesthetic agents. All animals were hyperventilated with oxygen after intubation until the experiments were terminated. Rectal temperatures were continuously observed and electrocardiograms were taken at frequent intervals as the dogs were cooled until cardiac activity ceased. No surgical procedures were carried out. Eight of 11 animals receiving cyclopropane, 2 of 10 receiving ether, and none of 10 receiving pentobarbital died in ventricular fibrillation. The mean rectal temperature of animals on cyclopropane or ether dying of ventricular fibrillation was 19°C. The mean rectal temperatures of all the deaths after the use of cyclopropane was 16.7°, after ether, 13.4°, and after pentobarbital sodium, 11.3°.

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Effect of Malonate and Antimycin A on Renal Tubular Transport of p-Aminohippurate.*† (22024)

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The dependence of renal tubular transport systems on certain energy producing reactions might be established by the use of specific metabolic inhibitors. There exists, for example, a correlation between the capacity of certain chemical compounds to inhibit the oxidation of succinic acid *in vitro* and to decrease the renal tubular excretion of p-aminohippurate (PAH) and phenolsulfonphthalein (PSP) *in vivo*(1). In the present report additional data are presented to support the hypothesis of Shideman and Rene(1) that energy derived from succinate oxidation (and Krebs cycle oxidations) is involved in the renal tubular mechanism which effects the excretion of PAH.

Materials and methods. Female, albino rats (150-200 g) of the Holtzman strain were anesthetized by the intraperitoneal administration of 35 mg/kg of pentobarbital and the right (control) kidney was removed. Cortical slices from this kidney were prepared and approximately 100 mg of these slices were placed in 20 ml beakers containing a volume of 2.7 ml of the medium employed by Cross and Taggart(2). The concentration of PAH, however, was half that used by these authors. The

TABLE I. Effect of Malonate Administration on Ability of Renal Cortical Slices of Rat to Accumulate PAH. Each animal received 1.2 ml of M sodium malonate subcutaneously 2 hr before and 0.6 ml of the same solution one hr before removal of the left kidney (6 animals).

	S/M concentration		
Rat No.	Before malonate (right kidney)	After malonate (left kidney)	% inhibition
1	8.42	2.43	80.8
2	7.81	3.59	62.0
3	6.07	2.27	75.0
4	8.39	4.07	58.5
5	10.10	8.19	21.0
6	14.90	6.27	62.1
	Mean ± S.E.		59.9 ± 8.5
			P <.01

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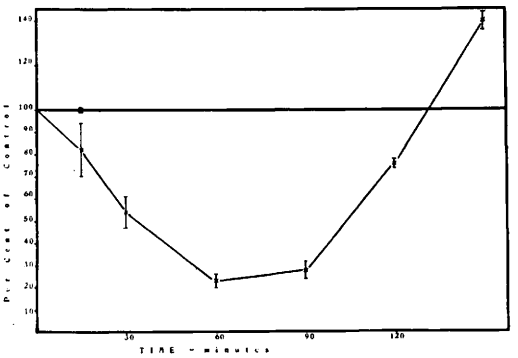

FIG. 1. Impairment of PAH accumulation by renal cortical slices from rats following administration of 1 g/kg sodium malonate. Each point with its standard error represents the data obtained from 6 animals. ×—malonate, □—saline, isosmotic with and equivalent in amount to malonate administered.

beakers were incubated for one hour at 25°C in a Dubnoff Metabolic Shaking Incubator with continuous oxygenation. Following incubation, the slices were removed from the medium, weighed, and the concentration of PAH in the slice and medium determined. The capacity of the cortical slices to accumulate PAH was expressed as the ratio of the concentration of PAH in the slice to that in the medium (S/M ratio). Following removal of the right kidney, as mentioned above, sodium malonate or antimycin A‡ was administered either subcutaneously, intraperitoneally, or intravenously and at varying periods of time thereafter the left kidney was removed from each of the test animals and the capacity of cortical slices to accumulate PAH was determined. Antimycin A was dissolved in either polyethylene glycol 200 or propylene glycol for injection and in 95% ethyl alcohol for addition to the medium in the *in vitro* experiments.

Results. Following subcutaneous administration of sodium malonate in the dosage indicated in Table I there is a significant decrease (59.9%) in capacity of the kidney to concentrate PAH. Dosage schedule of sodium malonate in this representative experiment is identical with that found by Busch and Potter(3) to inhibit *in vivo* metabolism of succinate by rat kidney. In other animals which received an equivalent amount of saline, isosmotic with malonate solution employed, there was no significant alteration in the capacity of cortical slices to accumulate PAH.

The reversible nature of the inhibitory effect of malonate on transport of PAH is apparent from the data presented in Fig. 1. Sixty minutes after administration of malonate the peak effect was observed. Inhibition of this renal excretory mechanism was not demonstrable for more than approximately 2 hours after administration of malonate. Two and one-half hours following administration of the inhibitor, there was noted an appreciable increase in capacity of renal cortical slices to accumulate PAH. Intravenous saline (isosmotic with malonate) produced no inhibition 15 minutes after administration. Sixty minutes after saline, however, there was observed a slight increase in the capacity of renal cortical slices to accumulate PAH. Since the S/M ratios were based on wet weight of renal tissue, the possibility existed that this slightly enhanced capacity to accumulate PAH might be due to alteration in water content of the kidney following injection of hypertonic saline. However, no significant differences in dry weights of renal slices before and after intravenous injection of hypertonic saline were found.

The influence of varying the dosage of malonate, administered intravenously, is illustrated in Fig. 2. At doses between 0.6 and 1.0 g/kg a linear relationship was found to exist between the intravenous dose of malonate

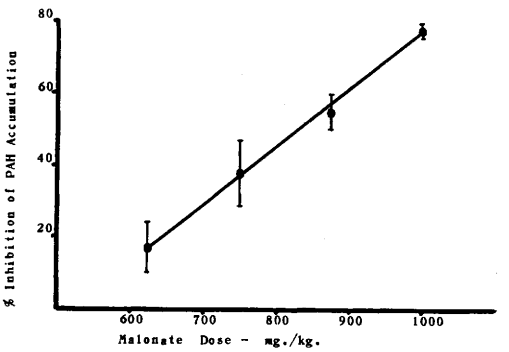

FIG. 2. Relationship between dose of sodium malonate and inhibition of PAH accumulation by renal cortical slices of the rat.

‡ Kindly supplied by Dr. F. M. Strong, Department of Biochemistry, University of Wisconsin, Madison.

TABLE II. Inhibitory Effect of Antimycin A on Accumulation of PAH by Renal Cortical Slice of the Rabbit. Slices were prepared from cortical portion of the kidney and incubated for one hr. Antimycin was dissolved in 95% alcohol and added in a volume of 0.1 ml to each flask. Slices incubated in a medium containing the same amount of alcohol but with no antimycin served as controls.

μg antimycin A/g tissue	% inhibition
2	5.8
4	13.5
6	43.8
8	63.5
10	68.5
100	82.6
1000	91.1
1500	97.4

and percentage inhibition of uptake of PAH.

Addition of antimycin A to a medium containing slices of renal cortex interferes with their capacity to concentrate PAH (Table II). These data are from a representative experiment in which the rabbit was employed to obtain sufficient tissue to determine the effects of a fairly wide range of concentrations of the inhibitor. Similar findings obtained in the rat. The succinoxidase activity of rat kidney is inhibited by approximately 2 to 7 μg of antimycin A per gram of tissue(4). In the same concentration range, antimycin A also inhibits the accumulation of PAH by renal slices of the rabbit.

In Table III are shown the effects of parenteral administration of antimycin A on accumulation of PAH by renal slices obtained from animals treated as described below. Administration of antimycin A produced marked inhibition of PAH uptake. Reif and Potter (5) reported that comparable doses significantly suppress succinoxidase activity in kidney of the rat. Animals receiving only propylene glycol intraperitoneally or 0.5% saline and polyethylene glycol 200 intravenously served as controls. These procedures produced no significant effect on capacity of cortical slices prepared from such animals to accumulate PAH. Administration of saline prior to the polyethylene glycol 200 was effective in preventing small but significant effects produced by the latter compound when it was injected intravenously.

Discussion. In previous studies metabolic inhibitors have been used to establish the dependence of a given physiological function on specific energy yielding reactions. 2,4-Dinitrophenol (DNP), a compound which is believed to uncouple aerobic oxidative and phosphorylation reactions, has been shown by Taggart and Forster(6), and Mudge and Taggart(7) to inhibit the renal tubular excretion of PAH, diodrast, and phenol red. Metabolic blockade at the level of the Krebs cycle, with either dehydroacetic or malonic acids(1) or inhibition of oxidative phosphorylation by DNP(7), does not indiscriminately influence all renal tubular functions. These observations suggest that there is some specificity in the allocation of energy derived from oxidative mechanisms and breakdown of phosphate bond compounds for the various renal tubular functions. Although metabolic inhibitors might produce a specific biochemical lesion *in vitro,* the interpretation that their *in vivo* action is a reflection of their *in vitro* effect might be questioned. Sodium malonate and antimycin A were chosen because of the evidence for correlation of *in vivo* and *in vitro* inhibitory effects. Busch and Potter(3) have presented evidence for the *in vivo* inhibition of succinate metabolism by malonate. The same concentration of antimycin A which Reif and Potter(5) have found to inhibit succinoxidase activity in the kidney, also produces an inhibitory effect upon the renal transport of PAH. Therefore, our data, correlated with those of the above authors, are highly suggestive that succinate oxidation (and Krebs cycle oxidations) functions *in vivo* as a source of energy for this transport mechanism.

Availability of energy derived from the oxidation of malonate for the transport of PAH

TABLE III. Effect of Antimycin A Administration on Accumulation of PAH by Renal Cortical Slice of the Rat. Following removal of control (right) kidney,(a) antimycin A (1 mg/cc in propylene glycol) was administered, after 40 min. the left kidney was removed or(b) 4 cc/kg of 5% saline was administered, followed in 30 min. by intravenous injection of antimycin A (1 mg/cc in polyethylene glycol 200) 15 min. after which the remaining kidney was removed.

Route	No. of animals	Dose, mg/kg	Inhibition of PAH accumulation (% ± S.E.)
Intraper.(a)	6	3	63.4 ± 10.5
Intraven.(b)	6	1	91.4 ± 1.5

has to be considered as a possible explanation for the capacity of the renal slice to accumulate the latter material 2½ hours after the administration of malonate. Busch and Potter (3) were able to recover only part of the malonate from the urine of animals receiving this compound. It is known that malonate can be oxidatively metabolized *in vivo*(8,9) and, therefore, may contribute to the pool of metabolites from which the excretory mechanism for PAH derives its energy. Furthermore, it is possible that malonic acid is metabolized or removed from the cell more rapidly than is succinate. Succinate (and other members of the Krebs cycle) which accumulate in the presence of malonate would be expected to be present in amounts greater than are usually found and supposedly would provide an additional source of energy for the transport of PAH. Whether or not either of these proposed mechanisms is correct remains to be determined.

Summary. The renal tubular excretion of PAH in the rat is inhibited by the *in vivo* administration of sodium malonate or antimycin A. The data here presented, when correlated with the results of other investigators, strongly suggest succinate oxidation can provide an *in vivo* source of energy for that renal mechanism which is responsible for the tubular excretion of PAH.

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Effects of Digitoxin on Exchangeable and Tissue Potassium Contents.* (22025)

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The effect of digitalis on both normal and abnormal myocardium is well known. Although it is an established fact that the glycosides have a direct effect on the heart, controversy still exists as to whether they have an *extracardiac* effect as well. Because the chemical structure of digitalis is similar to that of other steroid compounds which are known to affect salt and water metabolism—for example, sex and adrenal cortical hormones, it might be suspected that the glycosides would act similarly. There are, in fact, experimental evidences to suggest that these agents, under certain circumstances, act in a fashion similar to the adrenal cortical hormones(1,2).

A previous study(3) suggested that the intravenous administration of 0.2 mg of digitoxin per kg of body weight results in reversible shifts in the distribution of body fluids. The availability of radioactive potassium (K^{42}) has made possible the direct measurement of the exchangeable potassium con-

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