action. The therapeutic exploitation of these various pharmacological properties requires the appropriate form for administration. New compounds, synthesized by modification of chemical functional groups with a view to increased stability and solubility, must be compared with the parent substance in respect to the perseverance of the desired activity. In the present instance, we have converted adrenochrome into the stable and extremely water-soluble betaine hydrazone T.A.L. The clinical tests which we report show that the formation of the derivative has not destroyed the hemostatic effects of adrenochrome. These results, in addition to their practical interest *per se*, may serve as a guide in the study of the metabolic fate of the compounds; moreover, they point to the localization of the hemostatic effect in that part of the molecule not affected by the synthetic chemical modification.

Summary. Trimethylammoniumacethydrazone hydrochloride of L-adrenochrome (T.A.L.) is a potent hemostatic agent. It shares this property with the parent substance adrenochrome, but surpasses it by stability and solubility in aqueous solution. It may be injected intramuscularly in children in amounts of 10 mg without producing local reactions. A single injection of this amount shortens the bleeding time, as measured by Ivy's method, by an average of 38% in 32 subjects; except for 7 non-responsive cases with short pre-injection bleeding time, the mean bleeding time in the remaining 25 cases is cut in half. Only individuals with a short bleeding time fail to respond. Control tests show that the concomitant surgical intervention has no influence on bleeding time.

We are grateful to Dr. Sylvan E. Moolten, Director of Laboratories, St. Peter's Hospital, New Brunswick, N. J., for the preliminary clinical tests and for various suggestions, to Dr. Rudolph Kramer, Otolaryngologist to the Mount Sinai Hospital, for the facilities of his Service, to Mr. John Austin for the preparation of the compound and to Miss Aina Birnbaum for measuring bleeding times.

Received November 10, 1950. P.S.E.B.M., 1950, v75.

Effect of Maleic Acid on Renal Function. (18344)

ROBERT W. BERLINER,* THOMAS J. KENNEDY* AND JAMES G. HILTON.
(Introduced by A. B. Gutman.)

From the Columbia Research Service, Goldwater Memorial Hospital, and the Department of Medicine, Columbia University, College of Physicians and Surgeons, N. Y.

As part of an investigation of the capacity of certain organic acids to serve as effective buffers for the excretion of titratable acid, maleic acid was selected since its pK (about 6.5) is such as to be theoretically well suited to this function. It was observed, however, that when maleic acid was administered to an acidotic dog, the urine became alkaline despite the fact that the animal was in severe acidosis (plasma pH 7.0, CO_2 content 9.5 mM/1). Further investigations of the effects of maleate on renal function are the basis of the present report.

Methods and material. Trained, unanesthetized female dogs in the post-absorptive state were used in all experiments. Acidosis was induced by the administration of 5 g of NH_4Cl eighteen hours before some of the experiments and in others by the inclusion of dilute HCl in the infusion fluid. The technics and chemical methods used have been described in earlier publications from this laboratory(1,2). Maleate, generally at a dosage of 40 mg (0.35 mM) per kilo, was administered intravenously as a single dose in all experiments. The pH of the injected material

* Present address: National Heart Institute, National Institutes of Health, Bethesda, Md.

1. Berliner, R. W., Kennedy, T. J., Jr., and Hilton, J. G., *Am. J. Physiol.*, 1948, v154, 537.

2. Berliner, R. W., Kennedy, T. J., Jr., and Hilton, J. G., *Am. J. Physiol.*, 1950, v162, 348.

TABLE I. Effect of Maleate on Acidification of the Urine. Dog F, Weight 16 kg.

Time, min.	Creatinine clearance, ml/min.	Plasma CO_2 content mM/l	Urine pH	Urine Titratable† acidity, μEq./min.
−66	Start infusion: creatinine 0.4%, NaCl 50 mEq./l, Na_2HPO_4—NaH_2PO_4 (pH 5.8) 70 mM/l at 6 ml/min.			
−58 to −56	HCl (0.1 N) 50 ml. i.v.			
0- 20	68.5	15.5	5.85	136
20- 41	73.0	—	6.00	186
41- 56	76.7	16.1	6.00	203
58	Maleic acid—40 mg/k, i.v.			
56- 76	84.0	—	6.60	127
76- 96	66.5	13.9	7.22	2
96-116	57.0	—	7.38	0
116-137	63.2	10.6*	7.38	0

* Arterial plasma pH 7.26.
†All urines titrated to pH 7.40.

was usually brought to about 6.0 by the addition of $NaHCO_3$ to a solution of maleic acid.

Results. The most striking effect of maleate was on the acidity of the urine. In every instance, there was a sharp rise in the urinary pH observable in specimens obtained 15-20 minutes after injection of the maleate and persisting for a period of 3-4 hours after the single dose. The extent of the rise in urine pH was dependent in part on the severity of the acidosis. When the bicarbonate concentration of the plasma was 10 mEq. 1 or more, the pH of the urine generally rose to or above that of the plasma. In more severe acidosis, the pH of the urine increased, but frequently remained lower than that of the plasma. When the urine was alkaline before the injection of maleate, there was little or no further increase in urinary pH, but there was a further rise in the excretion of bicarbonate. An experiment illustrating the effect of maleate on the urine pH and titratable acidity is presented in Table I. In this experiment, the dog was rendered acidotic by the intravenous administration of HCl while urinary buffer was provided by the infusion of phosphate. The characteristic effect on urinary acidity in the face of moderately severe acidosis is shown.

Glomerular filtration was variably affected by maleate in the dosage used. In 18 of the 34 experiments, the creatinine clearance remained essentially unchanged or increased slightly. In 6, there was a drop of 10-20% and in 10 experiments, the creatinine clearance fell 20-50%. Nine of the 10 marked falls in filtration rate were observed in experiments in which glucose was also administered, for the measurement of glucose Tm (8 exp.) or as the diluent for the infusion (1 exp.).

Tubular capacity for the secretion of p-aminohippurate was measured in 5 experiments. In 4 of these, the Tm_{PAH} was found to be depressed an average of 15% (mean of all clearance periods after the maleate injection). In one additional experiment, the only one in which the PAH was administered in glucose solution, there was a fall of 55% in Tm_{PAH} along with a marked drop in creatinine clearance. An experiment showing the effect on PAH transport is shown in Table II, along with data to indicate the typical changes in electrolyte excretion following the administration of maleate.

The major effects of maleate on electrolyte excretion were those associated with impairment of the mechanism of acidification and bicarbonate reabsorption. Most of the increase in cation loss was due to the rise in sodium excretion; potassium excretion increased to a moderate extent.† No specific effect on the excretion of chloride was apparent. The excretion of endogenous phosphate

† Potassium secretion did not appear to be inhibited in an experiment in which maleate was administered during the infusion of potassium salts.

TABLE II. Effect of Maleate on PAH Transport and Excretion of Electrolyte. Dog B, Weight 20 kg.

Time, min.	Urine flow, ml/min.	Creatinine clearance, ml/min.	Tm_{PAH} mg/min.	Plasma HCO_3^- mEq./l	Urine pH	Rate of excretion: Na^+ μEq./min.	K^+ μEq./min.	Cl^- μEq./min.	HCO_3^- μEq./min.	PAH^- μEq./min.	$H_2PO_4^-$ $HPO_4^=$ μM/min.
−33 to −31	Creatinine 1.75 g, PAH 2.0 g, i.v.										
−30	Start infusion creatinine 0.45%, PAH 1% in water at 6 ml/min.										
0- 17	0.9	77	12.6		5.20	85	93	0	0.3	246	0.9
17- 38	0.4	64	12.5	20.3	5.12	40	82	0	0.1	212	—
38- 63	0.9	69	11.3		5.09	24	89	0	0.1	222	0.6
67	Maleic acid 40 mg/kg, brought to pH 6.5 with $NaHCO_3$, i.v.										
63- 86	2.0	68	12.3		5.72	134	115	10	1.1	214	—
86-110	3.5	80	13.4	19.9	6.00	273	156	0	4.5	250	11.7
110-133	5.7	74	10.4	20.5	6.90	288	151	0	38	224	—
133-153	7.2	73	10.5	20.5	6.97	292	131	0	53	221	17.6
153-173	6.2	74	8.6	19.4	7.18	331	117	0	76	222	—
173-193	7.3	75	9.8	19.9	7.37	425	128	0	133	232	39.4

generally showed a moderate increase after the injection of maleate.‡ There was a marked excess of measured cation over anion in the periods following the injection of maleate. Attempts to identify the anion responsible were unsuccessful. The total maleate injected was insufficient to account for the excess anion excretion and the titration curve of the urine did not suggest the presence of appreciable concentrations of maleate. Quantitative measurements showed that the change in anion excretion was not attributable to organic or inorganic phosphate, inorganic sulfate, citrate, or α-amino acids. Qualitative tests for dicarboxylic and keto acids were negative.

In nine experiments in which glucose Tm was measured, glucose reabsorption fell more or less markedly after maleate was administered. Interpretation of these results was made difficult, however, by the marked drop in the rate of glomerular filtration that almost invariably occurred. The decrease in glucose reabsorption was always greater, proportionately, than the change in creatinine clearance.

Toxicity. As is apparent from the effect on creatinine clearance of maleate during the infusion of glucose solutions, this combination of substances, for undetermined reasons, was considerably more toxic than maleate alone in the same dosage. Although animals given maleate alone showed transient impairment of renal function, all promptly recovered and showed no persistent impairment of health or renal function despite repeated dosage. Several of the dogs given glucose and maleate together died 2-3 days later and others showed marked elevation of the blood urea nitrogen and marked acidosis lasting for 1-2 weeks afterward. Post-mortem examination of animals that died showed diffuse necrosis of the convoluted tubules. No pathological studies were done on animals which did not receive both glucose and maleate as all such animals

‡ In experiments with infused phosphate, the calculated capacity to reabsorb phosphate decreased an average of 28%. In our hands, a stable reabsorptive capacity for phosphate has not, however, been demonstrable under other circumstances.

survived.

Effect on acid secretion by the frog gastric mucosa. The effect of maleate on acid secretion by the frog gastric mucosa was studied by the technic of Patterson and Stetten (3). Because of the buffering capacity of maleate in the physiological range of pH, it was not possible to measure the secretion of H^+ ions by the stomach into a solution containing appreciable amounts of maleate and the maleate was, therefore, added only to the chamber in contact with the external surface of the mucosa. No inhibition was evident with maleate concentrations of 100 mM/l. There may be some question of the penetration of the maleate to the region of acid formation under the circumstances of this experiment. NaF, 10 mM/l, was completely inhibitory when added under similar conditions.

Discussion. Maleate appears to exert an effect on acidification of the urine which is unique among substances whose action has been reported. Sulfanilamide impairs the excretion of titratable acid(4), but the effect is relatively slight and can be demonstrated only when acidosis is relatively mild. Cyanide (5) and tartrate(6) have been found to abolish urinary acidification but in these instances the effect is observed along with almost complete loss of renal tubular activity and a loss of the ability to excrete a urine with a pH different from that of the plasma. The latter is not the case with maleate since the capacity to excrete an alkaline urine is retained; urines with pH over 7.8 have been observed repeatedly. Although maleate appears to depress most of the renal functions studied to some extent, the effect on acidification is far greater than its other actions.

Maleate has been observed to exert an inhibitory action on a number of enzyme systems studied *in vitro*(7-11). It is not clear through which, if any, of these systems the effects described here are exerted.

Summary. Maleate administered to severely acidotic dogs at a dosage of 0.35 mM/kg abolishes the capacity to excrete an acid urine. Lesser effects on other renal functions are produced.

3. Patterson, W. B., and Stetton, DeW., Jr., *Science*, 1949, v109, 256.

4. Pitts, R. F., and Alexander, R. S., *Am. J. Physiol.*, 1945, v144, 239.

5. Nicholson, T. F., *Biochem. J.*, 1949, v45, 112.

6. Nicholson, T. F., Urquhart, R. W. I., and Selby, D. L., *J. Exp. Med.*, 1938, v68, 439.

7. Morgan, E. J., and Friedmann, E., *Biochem. J.*, 1938, v32, 733.

8. Morgan, E. J., and Friedmann, E., *Biochem. J.*, 1938, v32, 862.

9. Hopkins, F. G., Morgan, E. J., and Lutwak-Mann, C., *Biochem. J.*, 1938, v32, 1829.

10. Weil-Malherbe, H., *Biochem. J.*, 1938, v32, 2257.

11. Friedmann, E., Marrian, D. H., and Simon-Reuss, I., *Brit. J. Pharmacol. and Exp.-Chemotherapy*, 1948, v3, 263.

Received November 10, 1950. P.S.E.B.M., 1950, v75.

Biosynthesis of Radioactive Allantoin.* (18345)

J. D. VALENTINE, S. GURIN, AND D. W. WILSON.

From the Departments of Physiological Chemistry and Medicine, School of Medicine, University of Pennsylvania, Philadelphia.

Heinrich and Wilson(1) reported experiments dealing with the carbon precursors of the tissue purines of the rat. Since allantoin is one of the main end products of purine

* Aided by a grant from the American Cancer Society administered by the Committee on Growth of the National Research Council. The C^{14} used in this investigation was obtained on allocation by the United States Atomic Energy Commission.

1. Heinrich, M. R., and Wilson, D. W., *J. Biol. Chem.*, 1950, v186, 447.