Effects of Desoxycorticosterone and Adrenocorticotrophic Hormone on Mercurial Diuresis.* (19601)

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Certain adrenal cortical hormones decrease renal sodium excretion while mercurial diuretics increase sodium excretion. Axelrod *et al.*(1) have claimed that in dogs the administration of a large dose of ACTH inhibits or markedly reduces a mercurial diuresis. If an antagonism between mercurials and the electrolyte-retaining adrenocortical hormones could be demonstrated this phenomenon would be of help in interpreting the action of the mercurial diuretics. It was thus of interest to investigate a possible antagonism between mercurial diuretics and the salt retaining adrenal hormones. Our present experiments include a study of the possible antagonism between the mercurial mersalyl and adrenocorticotrophic hormone (ACTH), desoxycorticosterone acetate (DOCA)† and desoxycorticosterone glycoside (DCG).‡

Methods. Dogs weighing between 12 and 22 kg were used in these experiments. In some of the dogs the action of the mercurial before and after desoxycorticosterone treatment was studied in the unanesthetized animal. Another series of experiments was conducted under pentobarbital anesthesia. The animals received intravenously a constant rate infusion of 0.86% sodium chloride or 3% glucose which contained adequate amounts of para-aminohippurate (PAH) and inulin or creatinine. Renal plasma flow was determined by the PAH clearance at low plasma concentrations while glomerular filtration was measured by the inulin or creatinine clearance. Sodium and potassium in plasma and urine were measured by means of an internal standard or a Beckman flame photometer. Chloride in plasma and urine were determined by the method of Van Slyke and Hiller(2). In the unanesthetized dogs blood specimens were obtained from a jugular vein while in the dogs under anesthesia arterial blood was obtained from the femoral artery by means of an inlying needle. The general procedures used in these experiments have been described previously(3). The mercurial used was mersalyl‡ which was given as the sodium salt or in combination with cysteine (1:1 molar ratio).

Results. Three groups of experiments were conducted. *Group 1.* Three unanesthetized female dogs received 3.2 to 3.6 cc of 3% glucose per m^2 of body surface per minute. After control periods of about 90 to 120 minutes, mersalyl and cysteine (1:1 molar ratio) were given in a dose of 8 mg per kg over a period of 2 to 3 minutes. The resulting diuresis was followed for about 2 hours after the injection of the mercurial. The same animal then received daily an intramuscular injection of desoxycorticosterone acetate (DOCA) in oil in a dose of 0.5 mg per kg. After 4 to 6 days of DOCA administration, a second diuresis experiment was conducted under the experimental conditions described above. The action of the mercurial was essentially the same before and after DOCA administration. In Table I the data obtained on 3 dogs have been summarized. In all 3 dogs there was no inhibition of either the mercurial-induced diuresis, natriuresis or chloruresis by prolonged DOCA administration. The changes in potassium excretion were variable. In 4 of the experiments the mercurial increased potassium excretion while in 2 experiments potassium excretion per minute was decreased by mersalyl. In all dogs urinary potassium concentration fell after the injection of the mercurial. The administration of DOCA over a 4- to 6-day period did not significantly modify the mercurial in-

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TABLE I. Maximum Change in Sodium, Potassium, Chloride and Water Excretion Caused by Mersalyl before and after the Administration of DOCA in Unanesthetized Dogs (.5 mg/kg/Day). Mersalyl given intravenously 8 mg/kg.

Dog No.	Body wt, kg	Max increase in urine vol., cc/min Before DOCA	Max increase in urine vol., cc/min After DOCA	Max increase of excretion in: Na, mM/min Before DOCA	Na, mM/min After DOCA	K, mM/min Before DOCA	K, mM/min After DOCA	Cl, mM/min Before DOCA	Cl, mM/min After DOCA	DOCA given
1.	14	+4.5	+3.6	+.45	+.58	+.03	+.04	+.50	+.65	5 days
2.	18	+4.9	+5.8	+.64	+.60	+.08	—.05	+.68	+.62	6 "
3.	12.5	+5.6	+6.5	+.72	+.80	—.02	+.04	+.80	+.80	4 "

duced net changes in potassium excretion.

Group 2. Anesthetized dogs received 3% glucose infusions at a rate of 3.5 to 4 cc/min./m^2 of body surface. The dogs received 3 mg/kg of DOCA dissolved in ethyl alcohol intravenously. About 90 to 120 minutes later 30 mg/kg of mersalyl and cysteine in a 1:1 molar ratio was given intravenously. In 3 dogs studied in this way there was a definite diuretic response to the mercurial which was about equal to that seen in dogs which had not received DOCA. In another group of 3 animals desoxycorticosterone glycoside was given in a dose of 5 mg/kg intravenously and about 30 to 45 minutes later 30 mg/kg of mersalyl with cysteine was given by the same route. Here again the mercurial diuresis was observed and was about the same in the DCG pretreated and the untreated control experiments. In one animal pretreated with DCG as above, mersalyl, in a dose of 2 mg/kg was given into the left renal artery. The injected left kidney showed a marked increase in water and sodium excretion while the right or control kidney was practically unchanged (Fig. 1). It must be concluded that neither DOCA nor DCG given acutely have the ability to inhibit the diuresis produced by mersalyl.

Group 3. Four anesthetized dogs received 14 to 17 cc/min./m^2 of isotonic saline intravenously. These dogs received ACTH (Wilson Lot 79951) in a dose of 70 to 100 units intramuscularly. Within 2 hours a definite reduction in urinary sodium concentration was produced by the ACTH in 3 of the 4 dogs and now mersalyl was given together with cysteine in a 1:1 molar ratio in a dose of 30 mg/kg. The high dosage of mercurial was used to produce maximal effects on the kidney (3). Here again the mercurial produced a rise in urinary sodium concentration and an increase in sodium and water excretion. The results of one of 4 typical experiments are shown in Fig. 2. This experiment shows that the mercurial can counteract the ACTH induced reduction in urinary sodium concentration and that BAL counteracted the mercurial effects. The increase in sodium excretion was determined graphically by plotting sodium filtered against sodium reabsorbed before and after the mercurial. The depression of sodium reabsorption produced by the mercurial was estimated by the shift of the straight line re-

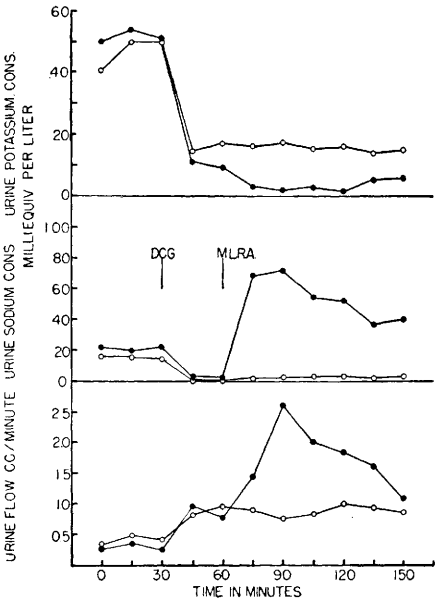

FIG. 1. Mersalyl diuresis in the desoxycorticosterone glycoside pretreated dog. Male dog 15.3 kg. Pentobarbital anesthesia. Ureters cannulated and excretion studies on each kidney. Infusion of 3.8 cc/min of a 3% glucose sol. Full circles = left kidney; open circles = right kidney. DCG: Intrav. inj. of 50 mg of desoxycorticosterone glycoside. MLRA: Inj. of 30 mg mersalyl into left renal artery for 2 min.

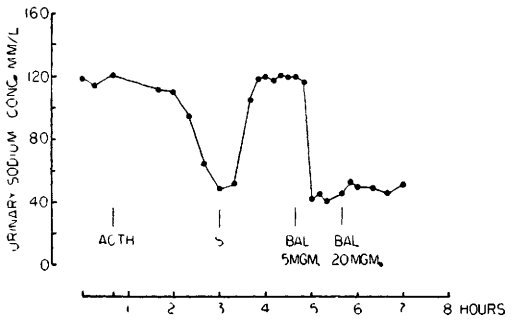

FIG. 2. Changes in urinary Na conc. following ACTH and mersalyl in the dog. Female 12.8 kg. Pentobarbital anesthesia. Isotonic saline infused 15.8 cc/min/m² body surface. ACTH: Intramusc. inj. 100 units ACTH (Wilson) S: 30 mg/kg of mersalyl with cysteine (1:1 molar ratio) intraven. BAL: 2,3 dimercaptopropanol intramusc. 5 and 20 mg/kg.

lating filtered to reabsorbed sodium. The details of this method have been described previously(3). By comparing the mercurial induced depression of sodium reabsorption with or without ACTH pretreatment the conclusion was drawn that ACTH in the dosage used and under our experimental conditions, did not alter appreciably the mercurial induced natriuresis and chloruresis (Table II).

Conclusion. Pretreatment of unanesthetized or anesthetized dogs with desoxycorticosterone acetate or desoxycorticosterone glycoside did not appreciably affect the diuresis induced by the mercurial mersalyl. In a similar manner ACTH in relatively large doses did not inhibit the mercurial diuresis. Our results indicate that a large dose of mersalyl actually produced a greater effect on tubular sodium reabsorption in the dogs receiving the ACTH. This increased excretion of sodium in the ACTH pretreated dogs is probably due to their initially lower urinary sodium concentration. The average values of the urinary sodium concentration at the time of the mersalyl injection was about 105 ± 12 for the control and 48 ± 6 milliequivalents per liter for the ACTH pretreated dogs. The peak increase in urinary sodium concentration following the mercurial was about 130 ± 4 and 135 ± 6 for the control and ACTH pretreated dogs respectively. It is of interest that the ACTH or DOCA induced reduction in urinary sodium concentration was readily counteracted by mersalyl. Mersalyl probably produced its renal effects by inhibiting some enzyme system essential for sodium reabsorption. Its ability to counteract the adrenocortical effects in the kidney may be due to an action of both these substances on the same enzyme system. However, mersalyl could conceivably produce the same results by acting on a sodium reabsorptive mechanism distal or proximal to the one acted on by the adrenocortical hormones. An action of mercury either distally or proximally could effectively mask the actions of the salt retaining

TABLE II. Depression of Sodium and Chloride Tubular Reabsorption Produced by Mersalyl on Control and ACTH Pretreated Dogs. A constant infusion of isotonic saline given intravenously at 15-17 cc/min in anesthetized dogs. Mersalyl given intravenously 30 mg/kg together with cysteine (1:1 molar ratio).

Exp. No.	Wt, kg	Depression of Na reabsorption	Depression of Cl reabsorption	
1	12.5	1.9	1.8	Controls
2	16	2.2	2.1	
3	14.8	1.9	2	
4	12	2	1.8	
5	17	2	2	
6	15.5	1.5	1.5	
7	15	1.4	1.5	
Avg		1.83±.36	1.81±.26	
1	14.8	2.6	2.5	ACTH (Wilson) given intramusc. 75-125 units about 2 to 3 hr before mersalyl was inj.
2	12.9	2.5	2.6	
3	16.5	2	2.2	
4	14	1.8	1.9	
Avg		2.22±.39	2.30±.32	

adrenal hormones without necessarily acting directly on the same renal tubular locus affected by the adrenocortical hormones. The results obtained with ACTH are different from those reported by Axelrod and MacLeod(1). Since no details regarding procedures and methods are available at this time, it is not possible to explain the differences in our results and those of Axelrod and MacLeod.

1. Axelrod, D. R., and MacLeod, J., *Fed. Proc.*, 1951, v10, 7.
2. Van Slyke, D. D., and Hiller, A., *J. Biol. Chem.*, 1947, v167, 107.
3. Farah, A., Cobbey, T. C., and Mook, W., *J. Pharm. Exp. Therap.*, 1952, v104, 31.

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Pyruvate Metabolism and Assessment of Semen Quality. (19602)

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During a study of the metabolism of pyruvate in mammary tissue it was observed that 2:4-dinitrophenol (DNP) stimulates the respiration without increasing the rate of utilization of the substrate, thus causing its more complete oxidation(1). Preliminary experiments in which the effect of DNP on the metabolism of pyruvate in spermatozoa was tested produced somewhat similar results(2). Since DNP is known to inhibit synthetic reactions(3) it was thought that it might be a useful reagent for an assessment of the quality of spermatozoa based on their synthetic power.

Experimental procedure. Washed bull spermatozoa(4) were suspended in phosphate saline(5), and incubated at 37° in the Warburg apparatus a) without addition, b) with pyruvate (0.005-0.01 M) and c) with pyruvate + DNP (10^{-4} M). All flasks also contained fumarate (0.002 M). The oxygen consumption was followed for one to 2 hours. Pyruvate and lactic acid were determined by chemical methods(6,7). Since pyruvate is rapidly reduced to lactic acid by spermatozoa under aerobic conditions(2), the amount of pyruvate metabolized by oxidative reactions is given by the difference between the amounts of pyruvate disappearing and lactic acid formed and is designated as "net pyruvate"(8).

Results. In early experiments in which semen from selected bulls of high fertility was used, the respiratory rate in the presence of pyruvate was often accelerated by DNP(2), but in samples from other equally fertile bulls, it remained unaltered or was slightly depressed. In all these experiments DNP increased the ratio O_2 consumed/net pyruvate. The fact that the observed ratios were relatively high and in the presence of DNP often exceeded the theoretical 2.5 suggested that the endogenous respiration was incompletely suppressed.

On addition of fluoride (0.02 M) the endogenous respiration of spermatozoa was reduced to a low level; although the level of respiration in the presence of pyruvate was also depressed, DNP now caused marked stimulation of the oxygen consumption in the presence of the substrate also in those samples in which, without fluoride, it was inhibitory (Table I). DNP did not stimulate the respiration in the absence of added substrates, or in the presence of small amounts of endogenous lactate (less than 5×10^{-4} M).

When the respiratory response to pyruvate and DNP of a larger number of samples was examined in the presence of fluoride, 5 groups could be distinguished. *A.* The respiratory rate in the presence of pyruvate was low, very little above the endogenous level; DNP caused large increases in O_2 consumption, often exceeding 100%. *B.* The rate of respiration in pyruvate was about twice the endogenous level and DNP caused little or no further increase. *C.* The respiratory rate in pyruvate was inter-