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Effect of Adrenal Steroids upon Plasma Volume of Intact and Adrenalectomized Dogs.* (30208)

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These experiments concern (a) the role of adrenal steroids in inducing alterations of the plasma volume of intact and adrenalectomized dogs following introduction by gavage of 250 ml of tap water or 0.9% NaCl into the gastrointestinal tract and (b) the effect of these steroids upon the plasma volume of intact animals subjected to prolonged food and water deprivation.

Methods. Fourteen adrenalectomized and 18 intact dogs were used. The adrenals had been removed for periods varying from several months to several years. During these intervals the animals were studied through cycles of insufficiency and recovery on substitution therapy. When used in the present experiments they were strong, vigorous animals, at peak weight and with normal blood chemistry, plasma volumes and arterial pressures. They were maintained by daily i.m. injections of 1 mg of desoxycorticosterone acetate in oil (DCA) plus 1 g of NaCl in their daily ration of fresh, ground, beef heart and puppy kibble. Before starting an experiment, steroid and salt supplements were withdrawn and control readings obtained for blood concentration and plasma volume. Food was withheld for the ensuing 24 hours, but unless otherwise stated, access to water was permitted to the time of steroid injection.

The free alcohols of the various steroids used were solubilized for i.v. injection by the method previously described(1). Saline was administered by stomach tube and followed within 5 minutes by i.v. injection of steroid. One and one-half hours after the last injection, blood samples were drawn. The 1.5-hour waiting period was arbitrarily chosen but since it proved satisfactory it was adhered to in all later experiments. No anesthesia was used at any time; the dogs were trained to remain quiet and relaxed during the necessary manipulations attending i.v. injections and blood sampling. The plasma volume method was that recommended by Gregersen and Stewart(2) and Chien and Gregersen(3). It has been utilized and adequately described by the present writers(4).

Results. Plasma volume changes following stomach tube administration of tap water or 0.9% saline to intact dogs. Nine dogs were used: 4 for testing effects of tap water and 5 for similar studies of saline. After giving the fluid the animals were placed in metabolism cages for 1.5 hours and then sampled at termination of this interval. The essential data were averaged (Table I A1). The plasma volume of dogs receiving tap water showed no appreciable alteration nor was any fluid voided during the test. The 5 animals given 250 ml of 0.9% saline responded quite differently. Plasma volume increased by an

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TABLE I. Plasma Volume Changes Induced Within 1.5 Hours Following Administration of 250 ml of Tap Water or Saline by Stomach Tube to Adrenalectomized and Nonadrenalectomized Dogs.

Steroid	Gavage fluid	Hb g%	Hct %	Plasma volume ml/kg	Plasma volume % change	No. dogs Data avg
		A. Nonadrenalectomized				
1. Control	0	13.3	39.5	54.2		
No steroid	tap water	13.6	43.3	52.9	— 2.4	4
2. Control	0	15.9	41.7	43.8		
No steroid	.9% NaCl	13.8	39.7	53.5	+22.1	5
		B. Adrenalectomized				
1. Control	0	15.7	40.2	42.8		
No steroid	.9% NaCl	14.1	36.9	42.1	— 1.6	4
2. Control	0	13.6	37.2	41.4		
Prednisolone 25 mg	tap water	13.9	34.8	39.0	— 5.8	2
3. Control	0	14.1	35.4	41.2		
Prednisolone 25 mg	.9% NaCl	11.6	30.9	48.7	+18.2	3
4. Control	0	16.0	43.7	47.0		
Desoxycorticosterone 25 mg	.9% NaCl	13.2	33.4	47.4	+ .8	3
5. Control	0	13.3	34.2	44.7		
Aldosterone 10 mg	.9% NaCl	11.8	30.9	43.5	— 2.6	2

+ Increase in plasma vol. — Decrease in plasma vol.

average of 22.1%, the largest individual rise was 25.7% and the smallest 17.6%. A decrease in hemoglobin accompanied the increase in plasma volume but the hematocrit remained unchanged (Table I A2).

Effect of administering 250 ml of saline or tap water, with and without accompanying steroids, upon plasma volume of adrenalectomized dogs. Saline in absence of glucocorticoid (prednisolone, Schering) or tap water accompanied by injection of 25 mg of prednisolone, failed to increase the plasma volume (Table I B1 and B2). The free alcohols of desoxycorticosterone and aldosterone (Ciba) also proved consistently ineffective for inducing changes in plasma volume (Table I B4 and B5, also Table II, 2 and 5). Despite the negative findings for plasma volume change, hemoglobin and hematocrit usually exhibited a modest decline, perhaps due to action of steroids upon the spleen presumably causing expansion of this organ with sequestration of erythrocytes. In sharp contrast to these negative results were the plasma volume increases which follow injection of 25 mg of prednisolone plus stomach tube administration of 250 ml of 0.9% saline. The plasma volumes of the 3 dogs so tested increased 18.2% and were associated with a marked fall in the hemoconcentration (Table I B3).

Effect of adrenal gluco- and mineralocorticoids upon plasma volume of intact dogs deprived of food and given no fluid for varying intervals. Prednisolone sodium succinate (Schering), 6-methyl prednisolone (Upjohn), and triamcinolone acetonide-21-phosphate (Lederle) were used. Each glucocorticoid was tested on 3 animals fasted 24 hours before using but allowed access to drinking water. All 3 steroids induced a plasma volume increase averaging 23.6% (Table II, 1). However, this same dosage of glucocorticoid given to dogs fasted for 48 hours and deprived of water for 24 hours did not raise the plasma volume. Evidently the starved animal requires much larger doses than 25 mg of prednisolone to affect the plasma volume. Hence massive doses of steroid were tested on 5 dogs given no food for 72 hours and no water for 48 hours. Prednisolone (175 mg) was injected i.v. in 7 divided doses of 25 mg each, distributed over the last 48 hours of the fasting period and blood samples drawn 1.5 hours after the last injection. Despite lack of food and water, pronounced hemoglobin and hematocrit changes occurred. The plasma volume had increased an average of 14.1 ml/kg or 28.2% by the end of 72 hours (Table II, 4).

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TABLE II. Effect of Intravenous Injection of Gluco- and Mineralocorticoids upon Plasma Volume of Nonadrenalectomized Dogs Deprived of Food and Water for Various Periods.

Time, hr	Hb, g%	Hct, %	Plasma volume ml/kg	Plasma volume % change	No. of dogs Data averaged
1. Glucocorticoids—25 mg i.v.* No food for 24 hr, water *ad lib.*					
Control	16.9	42.3	52.9		
1.5	14.2	39.8	65.4	+23.6	9
2. Aldosterone—25 mg i.v.* No food for 24 hr, water *ad lib.*					
Control	17.5	44.4	49.7		
1.5	17.8	44.4	48.2	— 3.0	1
3. Prednisolone—25 mg i.v.* No food for 48 hr, no water for 24 hours.					
Control	16.5	41.7	55.3		
1.5	16.9	40.8	53.4	— 3.4	6
4. Prednisolone—175 mg i.v.† No food for 72 hr, no water for 48 hours.					
Control	16.2	44.1	50.0		
48	14.2	39.6	65.1	+30.2	5
72	13.3	37.6	64.1	+28.2	
5. Aldosterone—20 mg i.v.‡ No food for 72 hr, no water for 48 hours.					
Control	14.9	41.5	50.5		
48	15.6	40.4	47.5	— 5.9	1
72	15.7	39.2	43.6	—13.6	

* Given as single injection 1.5 hr before sampling.
† Given in 7 doses of 25 mg each starting 24 hr after last feeding.
‡ Given in 5 doses of 4 mg each over 48 hr of food and water deprivation.

TABLE III. Plasma Volume and Volume of Distribution of Sodium Thiocyanate of 3 Fasted Nonadrenalectomized Dogs Receiving 125 mg of Prednisolone Sodium Succinate by Vein.* No food for 48 hours, no water for 24 hours.

Time	Body wt, kg	Hb, g%	Hct, %	Plasma volume ml/kg	Plasma volume Increase	Vol distribution NaSCN ml/kg	Vol distribution NaSCN Increase	Urine vol, ml/24 hr
Control	21.46	17.2	44.9	53.3		230		
24 hr	20.45	14.7	40.6	65.9	23.6%	276	940 ml	385

* Given in 5 divided doses of 25 mg over 24 hr. Data are averages. Free access to water permitted during first 24 hr of fast.

water-deprived animals raised the question as to the source of the fluid pouring into the blood. To test the fluid contribution, if any, from the extravascular-extracellular space the plasma volume and volume of distribution of thiocyanate were determined in 3 intact dogs. Each received no food for 48 hours and no water for 24 hours but 125 mg of prednisolone were given i.v. in divided doses over the final 24 hours of the fast. Plasma volume and volume of distribution of NaSCN were measured according to the analytical methods employed by Gregersen and his group(2,3). The analyses were made on 1 ml plasma using a Beckman Model B spectrophotometer with 10 mm cuvettes. The dye was read at 620 mμ, the thiocyanate at 460 mμ (Table III). The large amounts of prednisolone induced marked increases of plasma volume and simultaneous expansion of the thiocyanate space. The control averaged 230 ml/kg/dog but following injection of steroid the volume of distribution of SCN increased to 276 ml/kg/dog or an average of 940 ml despite elimination of 385 ml of urine during the 24-hour injection period.

Discussion. Marked slowing of intestinal absorption of certain electrolytes and water is characteristic of animals lacking adrenal cortices. This is evident even during the period of good health exhibited by these animals following withdrawal of salt or hormone therapy. The failing absorptive ability of the gut membranes can be restored to near normal functioning by replacement of the glandular secretions(5-8). In the present experi-

ments plasma volume increase was not elicited by forcing tap water to either intact or adrenalectomized dogs but the animals with adrenals readily increased their plasma volume when given 0.9% saline whereas the adrenalectomized dogs failed to show any such volume change. However, saline plus 25 mg i.v. of prednisolone, when given to the adrenalectomized dog promptly increased the plasma volume 18.2% within the 1.5-hour time limit set by these experiments. Thus, it seems that certain adrenal steroids in some way render the epithelial membranes lining the gastrointestinal tract more permeable to transfer of salt and water from gut lumen to blood thereby accelerating the absorptive process to the extent of sharply elevating the plasma volume. This hormone-stimulated increase in permeability of cellular membranes to salt and water is probably not confined to the gastrointestinal tract but may be general throughout the body. This is indicated by the experiments on the 72-hour fasted and 48-hour water-deprived intact dogs. Animals so treated and injected with large doses of adrenal steroid, rapidly raise the plasma volume 28-30% above control values within 1.5 hours of the last steroid injection. The substantial quantity of fluid shifted to the blood apparently was not at the expense of the water content of the extravascular-extracellular compartment since simultaneous determinations (using the same blood sample) of plasma volume and fluid available for dilution of thiocyanate showed that they increased together. It seems reasonable to assume that much of the fluid shifted to the blood, under influence of the hormone, to increase the plasma volume was contributed by the intracellular reservoir. The C_{11} oxysteroids or glucocorticoids appear to exert some kind of regulatory control over the active transport of certain electrolytes (presumably Na) and water across cellular membranes.

The failure of i.v. injections of solubilized mineralocorticoids, *e.g.*, desoxycorticosterone and aldosterone, to raise the plasma volume is not clear since it is well known that administration of DCA to either adrenalectomized(9) or intact dogs(10) for longer periods than employed in these tests, increases the plasma volume. Likewise, Addisonian patients treated with DCA and salt may exhibit striking elevations of plasma volume(11,12).

Summary. Twenty-four-hour fasted, intact dogs given 250 ml of 0.9% saline by stomach tube readily increase their plasma volumes due to rapid intestinal absorption of salt and water. Adrenalectomized dogs are unable to increase their plasma volume when subjected to similar treatment but promptly respond to saline plus adrenal steroids by rapid rise in volume. Intact dogs deprived of food and water for extended periods likewise markedly increase their plasma volume when given glucocorticoids by vein. Mineralocorticoids have no effect on the plasma volume of dogs receiving either tap water or saline orally during the brief time set by these experiments. The experiments indicate that certain adrenal steroids appear to be involved in the active transport of certain electrolytes (presumably sodium) and water across cellular membranes.

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