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Maintenance of Adrenalectomized Dogs with Low Plasma Volumes and Normal Electrolyte Patterns.* (28504)

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The plasma volume and electrolytes of intact and adrenalectomized dogs can be severely reduced and the animals maintained without apparent detriment to their activity and vigor(1-4). The present experiments are concerned with a simple method for drastically modifying the fluid and salt balance of adrenalectomized dogs by contracting their plasma volumes within a matter of minutes and keeping them low for 8-12 days with little or no deviation from normal of the plasma electrolyte patterns.

Methods. The adrenalectomized dogs were strong, active animals with normal arterial pressures, plasma volumes and plasma electrolytes. They had been operated for periods of 8 months to 1 year and table-trained for blood pressure and plasma volume studies. The dogs listed in Table I were maintained on 1 mg/day of desoxycorticosterone acetate (DCA) plus salt supplements in the ration. The oil-soluble steroid was omitted the day the experiments began and i.v. injections of 1 mg/day of the solubilized free alcohol of desoxycorticosterone (DOC) substituted.† There were 2 exceptions to this regimen: Dog 4, Table I, received 2 mg/day of DOC and Dog 5 was given 1 mg/day of aldosterone (Ciba). After securing control samples, the animals were injected i.v. with 300 mg of pentylenetetrazol (Metrazol, Knoll). Blood was drawn immediately after the severe reaction subsided and the plasma volume determined, using a standardized sample of the dye T-1824 and methods recommended by Gregersen and Stewart(5) and Chien and Gregersen (6), with a slight modification as to the time interval between samples.

A control blood sample was drawn from the jugular vein using a heparinized syringe; a solution of the dye, measured gravimetrically was then injected into the vein from an accurately calibrated syringe. This was cleared of all dye by rinsing repeatedly with blood drawn in and out of the barrel. Five ml samples were collected without stasis through an indwelling needle at 10, 13, 16 and 19 minute intervals after injection. To account for any residual dye which might be retained from previous determinations, the initial sample taken before dye injection was used to set the zero on the Beckman Model B. spectrophotometer employed for the determination. The analyses were made using 1 ml of plasma, 10 mm cuvettes and read at 620 mμ.

The animals were placed in metabolism cages, allowed free access to water but the food for Dogs 1-5 was given daily by stomach tube and consisted of 120 g of Lonalac

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† Solubilized desoxycorticosterone is prepared by dissolving the free alcohol of the steroid in hot, absolute alcohol and with constant stirring slowly adding, dropwise, propylene glycol followed by water. The final solution should contain 25% alcohol, 25% propylene glycol and 50% water. Aldosterone for i.v. use is prepared in the same way.

TABLE I. Reduced Plasma Volumes Associated with Normal Electrolyte Patterns in Metrazol-injected Adrenalectomized Dogs Receiving Mineralocorticoids (1 mg/day i.v.) and a Low Na Diet.

Days after Metrazol	Body wt (kg)	BP (mmHg)	Blood urea N (mg%)	Hb (g%)	Hmct (%)	Blood sugar (mg%)	Plasma vol (ml/kg)	Plasma electrolytes (meq/l) Na	Cl	K	Water intake (ml)
					1 mg DOC/day						
Dog 1											
Control	23.75	120	28	11.7	32.6	80	52.2	145	116	3.8	
Metrazol	22.91	90	32	13.7	38.2	89	34.7	150	117	6.1	
1-8	21.93	68	72	14.2	40.4	85	34.8	141	111	6.3	890‡
9*	21.81	110	36	11.3	33.3	98	46.7	133	106	5.2	920
Dog 2											
Control	18.72	122	20	11.1	30.4	72	50.0	145	117	4.1	
Metrazol	18.41	96	25	13.2	36.3	91	38.3	153	120	4.1	
1-7	17.12	73	32	13.6	37.5	76	37.3	142	114	4.6	679‡
8-12	17.01	69	55	13.4	37.2	89	37.4	139	115	4.6	718‡
13*	17.12	102	52	9.7	27.5	96	52.3	133	110	4.4	1090
Dog 3											
Control	26.14	110	33	12.5	36.0	77	43.2	140	115	5.0	
Metrazol	25.68	76	32	15.2	43.5	90	18.4	153	116	4.9	
1-4	24.77	58	94	17.3	44.9	81	21.0	132	92	6.1	755‡
5*	24.32	110	64	13.1	35.8	115	42.2	130	94	4.6	1170
Dog 4											
Control	20.68	122	23	14.0	40.2	82	45.0	147	115	4.6	
Metrazol	20.11	106	30	18.1	48.2	75	33.7	158	118	4.6	
1-8†	19.43	79	37	17.3	45.4	70	26.6	142	111	5.2	718‡
9*	19.09	107	19	15.2	40.4		50.5	140	106	4.1	1270
					1 mg Aldosterone/day						
Dog 5											
Control	18.86	109	29	12.2	35.5	74	42.1	141	112	5.0	
Metrazol	18.72	86	24	14.7	40.8	89	26.7	150	115	4.5	
1-9	18.03	65	41	13.8	36.7	87	29.7	140	107	4.9	614‡
10*	17.80	95	19	10.8	28.5	89	53.7	136	102	4.4	1140

* Prednisolone hemisuccinate given in 2 divided doses 24 hr before blood sample taken.
† Given 2 mg/day i.v. of solubilized desoxycorti osterone.
‡ Average daily water intake for number of days indicated in Column 1.

(Mead Johnson) suspended in 220 ml of distilled water. This low-sodium proprietary food contains 27% protein, 38% carbohydrate, 28% fat and minerals (ash) 5%, including Na 0.02% and K 1%. One hundred twenty grams supplies about 600 calories, 32 g of protein and 24 mg of Na. At termination of the experiments each dog was given an i.v. infusion of 200 ml of saline-glucose plus 100 mg of prednisolone (Schering).

Results. The immediate response to Metrazol was a drastic reduction of plasma volume, a moderate fall in the mean arterial pressure and some increase in hemoglobin and hematocrit. These changes were accompanied by a sharp, but fleeting rise in plasma Na which returned to control values within 5-7 minutes. Upon recovery from the Metrazol reaction and initiation of force-feeding of the low Na diet, the dogs were active, behaved normally and seemed to be in good health. However, after a few days changes slowly appeared; the weight declined, blood urea nitrogen and blood sugar rose, increased hemoconcentration was present. The plasma volume of all the animals remained at low levels. Dog 3 exhibited a very drastic plasma volume decline of 24.8 ml/kg. This quantity of blood disappeared from active circulation within a few minutes after injecting Metrazol. The dog later refused to submit to force-feeding without a prolonged struggle which resulted each time in regurgitation of the Lonalac. He failed rapidly despite ample DOC and ingestion of two-thirds of a liter of water a day. Dog 5, Table I, received by vein, 1 mg/day of aldosterone for 9 days but the results differed little from those obtained following DOC treatment. The plasma volume increased slightly but remained for 9 days 12.4 ml/kg below preshock level. The plasma Na did not fall below the normal

TABLE II. In Short Term Experiments Plasma Volumes of Metrazol-injected Adrenalectomized Dogs Remain Low Despite Adequate Supplies of Food, Water and Mineralocorticoids. (Average of 3 animals)*†

Hr after Metrazol	BP (mm Hg)	Hb (g%)	Hmct (%)	Plasma vol (ml/kg)	Plasma electrolytes (meq/l) Na	Cl	K	Water intake (ml/24 hr)
Control	116	15.0	40.0	42.4	144	115	4.3	681
Metrazol	97	15.5	40.0	26.1	158	121	4.8	
24	100	14.9	38.3	28.0	149	114	4.8	160
48	94	13.6	35.4	29.6	145	112	5.2	2193

* Food contained 1.4 g Na, 1.1 g Cl and 0.96 g K. Animals also received 2 mg/day DOC, plus 1 g NaCl and water *ad libitum*.

† Each dog injected i.v. with 300 mg Metrazol.

range until the 10th day after injection of Metrazol.

Three adrenalectomized dogs (Table II) were injected with 300 mg of Metrazol and thereafter received 2 mg DOC/day by vein and were fed their regular food ration of cooked beef heart, Ken-L-Ration and Kibble, plus 1 g of NaCl supplement and water *ad libitum*. The data reveal that after 48 hours on this feeding schedule the plasma volume of these dogs remained low, averaging 30% below control readings. Several days were required before the animals restored their plasma volumes to normal while on this regimen.

Discussion. The decline in plasma volume following Metrazol injection occurred with such rapidity that external loss of body water could be eliminated as a cause for the fall in plasma volume. Shift of water into cells owing to lowering of plasma electrolytes can also be ruled out as a contributing factor since these ions exhibited a substantial but evanescent increase under stimulus of the drug, then declined to preshock level within a few minutes and remained unchanged thereafter. It seems not improbable that the sudden decline of the plasma volume was due to rapid sequestration of large volumes of fluid and cells at the periphery of the vascular tree due to an intense and generalized vasoconstriction, initiated by the action of Metrazol upon the autonomic nervous system. Delgado(7) and Hoff *et al.*(8) cite numerous examples of autonomic responses and marked vascular reactions to direct stimulation of restricted regions of the cerebral cortex. It seems probable that a centrally acting, powerful, stressor agent such as Metrazol would also elicit strong reactions from this system, perhaps with release of vasoactive substances. In fact, Weil-Malherbe(9) and Montagu(10) report greatly increased concentrations of catecholamines in the plasma of patients subjected to electroshock or given subconvulsive doses of Metrazol.

Dogs with functional adrenal cortices quickly recover from the severe electro- and Metrazol-reaction(11,12) and restore their depleted plasma volumes to control levels within an hour or less. However, animals lacking these glands and subjected to identical treatment are unable spontaneously to increase the low volumes and must be given adrenal steroid to enable them to do so. They apparently are unable to relieve the vasoconstriction, which presumably persists and ends in circulatory failure due to interference with free flow of blood at the periphery, closing of large areas of the microcirculation, reduction of the plasma volume, marked slowing of the circulation and stagnant anoxia. Administration of glucocorticoids restores activity and vigor within a few hours without the animal having access to exogenous sources of food and water(13). These data afford no clue as to how recovery is brought about, other than assuming release of the vasoconstriction and initiation of free blood flow at the vascular periphery.

The adrenalectomized dogs fed the low Na diet and receiving high dosage of aldosterone and desoxycorticosterone, although unable to raise their low plasma volumes, were maintained in good condition for 8-12 days without the necessity for administering glucocor-

ticoids. Presumably, the mineralocorticoids plus the small amount of Na in the Lonalac, and the increased water intake, enabled the animals to maintain sufficient arterial pressure to ward off circulatory collapse and enough renal activity to prevent lethal accumulations of potassium during the 8-12 days of the experiment. If salt, water and DCA are withheld during the post-reaction period, both electro- and Metrazol-treated adrenalectomized dogs usually succumb within 24-36 hours unless glucocorticoids are administered(11,13).

The sharp but evanescent rise in plasma Na which is an invariable accompaniment of the electroconvulsive and Metrazol reaction in dogs, has been discussed previously(12).

Summary. Adrenalectomized dogs upon recovery from the severe convulsions following Metrazol injection can be maintained active and vigorous 8-12 days with low plasma volumes but with normal electrolyte patterns. This is accomplished by daily i.v. injections of desoxycorticosterone or aldosterone, a low Na diet and allowing access to water. The plasma volume falls immediately after Metrazol averaging 35.2% below preinjection levels and persists at low values. The mean arterial pressure slowly declines eventually necessitating administration of glucocorticoids. The plasma volume fall occurs so rapidly that fluid loss to the exterior must be ruled out as a cause for the low volumes. Shift of water into cells evidently does not take place since the plasma electrolytes, after an initial marked rise, rapidly decline to preinjection levels. The convulsant drug apparently induces intense vasoconstriction, evidently closing large areas of the microcirculation, trapping and pooling substantial quantities of blood at the vascular periphery. Presumably adrenalectomized, Metrazol treated dogs receiving adequate mineralocorticoids, a low Na diet and free access to water are unable to relieve the induced vasoconstriction and reestablish free blood flow in the small vessels of the circulatory tree in the absence of C_{11}oxysteroids.

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Chlorpromazine Effects on Pregnancy and Offspring in Mice.* (28505)

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Chlorpromazine has attained extensive clinical use as a tranquilizing agent for alleviating symptoms in emotionally disturbed subjects. Its reported psychotropic and antiemetic effects have also made it useful for controlling premenstrual tension and anxiety, nausea, and various complications of pregnancy(1,2). In view of its potentiation of a variety of anesthetic agents, chlorpromazine

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