Plasma Volume and Electrolyte Changes Induced by 2-Methyl-9 Alpha Fluorohydrocortisone in Fasted Adrenalectomized Dogs.* (23760)

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Hormones of the adrenal cortex appear to exert a profound regulatory control over the internal distribution of water and certain electrolytes(1-2). There are clear indications of fluid, Na, Cl and K shifts between intra- and extracellular body compartments following i.v. administration of potent analogs of hydrocortisone such as 2-methyl-9α-fluorohydrocortisone (2-methyl FF) and 1-dehydrohydrocortisone (1-dehydro F), to fasted adrenalectomized dogs recovering from severe insufficiency. Further evidence supporting this view of adrenal cortical function has been obtained by simultaneous determinations of plasma volume and electrolyte changes in healthy adrenalectomized dogs receiving the potent corticoid 2-methyl FF while deprived of food and water.

Methods. Sixteen adrenalectomized dogs were used; in the interval between experiments they were maintained in good health by daily i.m. injections of 0.5 mg DCA in oil, plus 2 g of salt supplements in the food. The extra salt was withheld 3 days before an experiment and the dogs were fed a ration which allowed them a total daily intake of 1.47 g of Na and 0.94 g of K. Twenty-four hours after the last feeding, blood samples were taken, the bladder drained by catheter and the animals placed in metabolism cages. Food and water were then withheld for 48 hours. The control data obtained are designated "initial" on Table I. The dogs received no food for 24 hours before sampling but had access to water hence they were 72 hours without food at termination of the experiment. Intravenous injections of 2-methyl FF were administered daily in divided doses (Tables I and II). Free alcohol of 2-methyl FF was injected in 25% absolute ethanol, 25% propylene glycol and 50% water. The compound was dissolved in hot alcohol and with constant stirring the hot propylene glycol was added dropwise. This was followed by hot water delivered in the same manner. These procedures were necessary because of the relative insolubility of the compound in an aqueous medium. The method for plasma volume determination was essentially that of Chinard(3), using a standardized sample of the dye T-1824. After withdrawal of a dye-free blood sample from the jugular vein employing a heparinized syringe, a measured sample of the dye was injected into the jugular from an accurately calibrated syringe, the syringe being cleared of all dye by rinsing repeatedly with blood. Samples were collected from this vein without stasis through an in-dwelling needle at 10, 13, 16 and 19 minutes after injection. In order to account for any residual dye which might be retained from the previous determination, the initial sample, taken before dye injection, was used to set the zero on the Beckman Model B spectrophotometer employed for the determinations. All values for plasma volume were calculated from semi-log plots(4). Quantity of blood withdrawn for electrolyte and plasma volume study was 37 cc for each of the 3 samples, or a total of 111 cc removed over 48 hours. The plasma loss was compensated for by i.v. injections of an equivalent amount of 5.5% glucose, calculated from the hematocrit. Two "control" dogs were simply continued on the routine maintenance therapy of daily i.m. injections of 0.5 mg DCA without other steroid treatment during the fasting period. Each of these dogs was also injected twice daily i.v. with 7.5 cc of the vehicle used for solubilizing the 2-methyl FF.

* Acknowledgement is made for financial and other valuable aid: The Upjohn Co., Ciba Pharmaceutical Products Co., Merck, Sharp & Dohme, N. J. Heart Assn., Nat. Science Fn. and Nat. Inst. of Arthritis and Metabolic Diseases, USPHS.

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TABLE I. Plasma Volume and Hemoconcentration Changes Induced in Fasted Adrenalectomized Dogs by 2-Methyl FF.

No. of dogs averaged	Dose, mg/day	Hr bled	Body wt, kg	BP, mm Hg	Blood urea N, mg %	Hb, g %	Hmct, %	RBC 10^6, mm^3	Blood sugar, mg %	Plasma vol, cc/kg
					2-Methyl FF (free alcohol)					
5		Initial	14.8	114	11.0	12.9	35.0	4.67	80	47.1
	1	24	14.1	117	10.1	11.2	31.6	3.85	86	55.2
	1	48	13.7	113	8.8	10.1	29.3	3.26	90	62.7
2		Initial	20.8	114	18.2	11.8	32.8	5.47	80	48.5
	3	24	19.6	114	10.9	9.9	26.9	4.59	78	64.5
	3	48	19.0	98	9.4	9.0	27.7	3.38	85	66.5
2		Initial	18.7	99	22.5	13.4	34.4	5.12	77	60.5
	4.5	24	17.6	107	19.1	11.1	31.7	4.04	85	70.8
	3	48	16.9	102	13.4	9.6	29.5	3.57	82	73.5
3		Initial	14.4	113	20.6	12.2	34.4	4.78	86	59.0
	5	24	14.1	111	14.8	10.7	29.4	4.14	95	50.0
	5	48	13.6	121	13.1	10.5	29.4	4.22	96	63.0
2		Initial	12.7	108	16.6	15.5	39.8	5.63	85	40.1
	8	24	11.7	93	12.2	12.1	33.4	4.87	95	47.4
	8	48	11.3	93	14.4	10.4	32.2	3.99	103	53.5
					Desoxycorticosterone acetate-maintained "controls"					
2		Initial	15.1	107	18.8	10.3	29.8	4.24	81	50.5
	.5	24	14.4	115	19.3	10.7	28.3	5.03	70	45.2
	.5	48	14.0	105	17.3	10.1	27.8	4.62	72	47.5

Hematocrits of dogs were determined by placing the blood in capillary tubes in an air turbine rotated at 20,000 g for 5 minutes, and plasma and urine Na and K concentrations were measured by the Perkin-Elmer flame photometer, Model C. It should be emphasized that the animals used in the experiments were healthy, vigorous dogs which showed no signs of adrenal insufficiency either before or after completion of these studies.

Results. The data regarding mobilization and internal redistribution of electrolytes and water in the fasted dogs following i.v. injections of 2-methyl FF, are presented in Tables I and II. All of the figures in Table I represent averages of data from animals grouped according to dosage of steroid they received. The dogs lost between 0.8 and 1.8 kg in

TABLE II. Correlation of Na, Cl and K Excretion with Plasma Volume and Plasma Electrolyte Changes in 14 Adrenalectomized Dogs Receiving 2-Methyl FF during a 48-Hour Fast.

Total dosage 48 hr, mg	Max plasma electrolyte changes, meq/l			Total urine electrolyte excr., meq/48 hr			Max plasma vol changes		Total urine vol, cc/48 hr
	Na	Cl	K	Na	Cl	K	cc/kg	%	
2	+ 6.3	+2.8	- .88	17.20	28.94	18.82	+19.2	+50.3	440
2	+13.0	+6.2	- .49	6.07	29.00	27.20	+23.5	+52.9	325
2	+ 2.5	+1.0	-1.19	11.82	24.23	23.93	+ 6.5	+12.1	530
2	+ 6.5	+2.0	-1.28	23.86	38.37	28.71	+17.3	+32.4	760
2	+ 4.5	- 4.1	-1.30	4.85	17.72	18.30	+11.1	+24.3	335
6	+ 5.5	+3.5	-1.18	7.16	41.67	10.70	+23.0	+44.2	355
6	+ 5.0	+4.0	-1.46	28.67	33.46	44.97	+15.0	+33.3	1050
7.5	+13.2	+6.5	-1.06	18.93	28.89	39.83	+14.8	+23.9	515
7.5	+ 7.5	+2.8	- .45	21.35	45.00	33.77	+26.2	+44.2	1125
10	+11.5	+4.0	- .43	27.92	40.19	15.21	not determined		370
10	+11.0	+8.1	- .57	53.99	64.39	23.62	"	"	671
10	+ 7.5	+1.0	- .60	9.35	21.48	17.93	+ 4.0	+ 6.8	325
16	+ 6.0	- 2.9	- .90	5.49	11.17	19.49	+14.4	+40.4	403
16	+ 4.0	- 4.9	- .88	6.48	10.42	26.05	+12.4	+27.8	428
				Desoxycorticosterone acetate-maintained "controls"					
1.0	- .5	- 2.4	+ .06	41.92	38.12	15.16	- 4.1	- 7.9	567
1.0	- 3.5	- 3.6	+ .74	42.78	35.18	16.83	- 6.4	-12.9	288

+ = Increase in electrolyte or plasma vol. - = Decrease in electrolyte or plasma vol.

weight during the experimental period. The blood urea nitrogen was sharply reduced and the hemoglobin changes indicated marked blood dilution. The initial hemocrits are low due in part to the anemia characteristic of long-term adrenalectomized dogs. However, following injection of the steroid, the packed volume of red cells showed a decrease from the control level. Likewise the reduction in erythrocyte count furnished further evidence that extravascular fluids were entering the blood. Blood glucose increased appreciably during the fast, no doubt owing to the strong glucocorticoid effect of 2-methyl FF. Plasma volume increased by 24-53% in 10 of the 12 steroid-treated dogs in which it was measured. The 2 remaining dogs showed smaller rises of about 7 and 12%. There was, curiously, no suggestion of dose-response effects within the dose range used. The elevation of plasma volume was accompanied in all cases by simultaneous increases in plasma Na concentration. Chloride concentration changes were of lesser magnitude and sometimes in the opposite direction than those of sodium. Plasma K concentration invariably declined (Table II). Electrolyte and plasma volume changes in the 2 fasted control animals continued only on maintenance doses of DCA, were the reverse of those following administration of 2-methyl FF. The plasma volume declined approximately 8-13% but the plasma Na and Cl concentration failed to rise. Blood sugar showed a moderate decline during the fast, as was anticipated, since DCA lacks glycogenic activity.

Discussion. Elevation of the plasma volume was associated with a well defined rise in plasma Na and to a lesser extent with that of Cl. A fall in Na and Cl should occur in the face of an increasing volume of fluid in circulation unless additional electrolytes from some extravascular depot were entering the blood stream. Evidently the steroid induced an outflux of Na and Cl from some as yet ill-defined source for despite expansion of plasma volume, the rise in plasma Na for example, ranged from 2.5 to 13 mEq/l in starved dogs whose plasma volume simultaneously increased 6.8 to 52.9% above control values (Table II). The excess fluid shifted into the blood presumably came from intracellular sources since exogenous supplies were not available. The "extra" Na which appeared in the plasma and urine of the hormone-treated dog can probably be accounted for by transfer of the cation from the mass of body cells which are known to contain small quantities and perhaps especially from bone and collagenous tissues both of which are considered to be rich in labile Na(5-7). The hormone seems to be particularly active in inducing shifts of intracellular K to the extracellular (and intravascular) compartment and thence to the kidney for elimination. The K diuresis resulting from injection of 2-methyl FF appears to be more pronounced in fasted adrenalectomized dogs recovering from insufficiency than in healthy animals adequately maintained on DCA(1-2). Apparently an undue accumulation of K collects within the cells in the absence of cortical hormones, which the dog is unable to release for renal excretion until the hormone deficit is corrected. Relief of symptoms is closely associated with and apparently dependent upon outflux of water from the cells.

Summary. Adrenalectomized dogs maintained in good health on 0.5 mg DCA/day were deprived of food and water for 48 hours and repeatedly injected with the analog of hydrocortisone, 2-methyl-9α-fluorohydrocortisone. Following injection simultaneous increases of plasma volume and plasma Na occurred along with hemodilution and decrease in plasma K and blood urea nitrogen. The animals continued to excrete Na, Cl and K during the fast, leading to further loss of water and electrolyte. The hormone apparently induced an outflux of fluid and electrolyte from unidentified sources but which are presumed to be cells, bone and collagenous tissues. The experiments afford further evidence that the adrenal cortex elaborates hormones which exert regulatory control over internal distribution of water and certain electrolytes between fluid compartments of the body.

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Received December 5, 1957. P.S.E.B.M., 1958, v97.

Potassium Acetate Inhibition of *Lactobacillus casei*. II. Strain Differences. (23761)

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It was reported in the first paper of this series(1) that *Lactobacillus casei* 7469 is markedly inhibited by the dual but not separate action of potassium and acetate ions. The inhibition was completely reversed by either lithium or sodium ions and by various fatty acids(1). Occasional failures of potassium acetate to inhibit *L. casei* growth were encountered during the course of this work but were not reported(1) because it appeared then that they were due merely to technical difficulties. It has now been found, however, that strains of *L. casei* differing markedly among themselves in sensitivity to potassium acetate are derivable from our stock culture, and it seems likely on the basis of these findings that our earlier variable results were caused by fortuitous population differences in the experimental inocula.

Methods. The stock culture of *L. casei* 7469 employed in these studies has been maintained in this laboratory since 1943 as described previously(2), with the exception that since 1948 fresh cultures have been prepared only every 3 weeks (originally this was done every 2 weeks). The possibility of accidental contamination during this period was kept at a minimum by preparing 2 or more stock cultures at each transfer time. Successive stock transfers were made only from the original, unused culture in each case, whereas transfers to experimental media were made exclusively from duplicates of this culture. The stock culture was never purified because it seemed likely that frequent and random selection of single cells for this purpose might lead to early divergence of originally identical strains in different laboratories. The *population characteristics* of this *L casei* culture with respect to potassium acetate inhibition were determined as follows. Cell suspensions prepared by transferring growth from the stock culture (using a sterile wire) to sterile saline solution were diluted appropriately and plated out in the same medium as that used in maintaining the stock culture (Yeast Dextrose Agar-Difco). The plates were incubated 40 hours at 35° under carbon dioxide, and growth from isolated colonies was transferred either directly to liquid inoculum medium or (by stab) to Yeast Dextrose Agar. Inocula prepared either directly from colony growth or from the stab cultures derived from such growth were tested by the procedures employed previously(1).

Results. Screening of approximately 1000 freshly isolated cultures in several independent experiments over a 3 months period revealed that from 6% to 45% of the isolates in a given series were resistant to inhibition by potassium acetate. The remaining isolates were not only highly sensitive to potassium acetate, but failed to grow normally even at the usual relatively low levels of potassium and acetate unless provided with a supplement of DL-lactate (the effects of sodium and lithium ion(3) were not determined in

* Paper 123. This work was aided by grants from National Multiple Sclerosis Soc., U.S.P.H.S., and University of California. The authors are indebted to Evelyn Brown for technical assistance.