should be looked for in such patients, since, if present, its correction might prolong life so that measures directed toward the underlying process of endotoxin shock could be effective.

Summary. An immediate and progressive fall in blood glucose level occurs in CCl_4-treated guinea pigs given a lethal dose of bacterial endotoxin. The hypoglycemia is evidently due to a decrease in rate of hepatic glucose production. Maintenance of the blood sugar level appears to prolong survival but does not alter the extreme susceptibility of these animals to endotoxin. Hypoglycemia should be looked for in patients with severe liver disease who develop gram-negative bacteremia, since if uncorrected it could render all other therapeutic measures ineffective.

The authors are indebted to Dr. Walter Mertz for advice and for critical review of this manuscript.

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Received December 9, 1963. P.S.E.B.M., 1964, v115.

Relationship of Plasma Aldadiene Levels and Antimineralocorticoid Effects of Spironolactone in the Laboratory. (29052)

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Oral administration of spironolactone,* a steroidal spirolactone(1) and an antagonist of the renal electrolyte effects of mineralocorticoids(2), produces measurable plasma concentrations of a fluorogenic, Δ^6-metabolite (3), 3-(3-oxo-17β-hydroxy-4,6-androstadien-17α-yl)propanoic acid lactone(1) or aldadiene. Such data on plasma aldadiene have been applied in study of gastrointestinal absorption with various pharmaceutical preparations of spironolactone(3-7). In both experimental animals and man, data have suggested a correlation of aldadiene titers with renal electrolyte manifestations after spironolactone administration(4,7). However, as aldadiene obtained synthetically also possess antimineralocorticoid properties(1,8), an important question relates to relative influence of the Δ^6-metabolite on renal properties of spironolactone. As one approach, therefore, spironolactone and aldadiene were examined comparatively in rats and dogs for plasma aldadiene levels and urinary electrolyte effects; some data believed to be pertinent to the question are summarized below.

Material and methods. In rats (175-225 g), spironolactone or aldadiene was orally administered concurrently with 12 μg of deoxycorticosterone acetate (DCA) subcutaneously, as solutions of corn oil. Four-hour samples of urine were collected in metabolism cages (4 rats/cage) in one series of animals for Na and K determination. Antimineralocorticoid activity of the spirolactones was assessed by reversal of the urinary Na/K response to DCA according to previously described procedures(2). DCA alone induces Na retention and K loss, to produce thereby a reduction in the ratio of urinary Na/K. Other rats treated identically were sacrificed under ether at 1, 2, 3 and 4 hours after treatment, and blood specimens from the abdominal aorta collected in heparinized tubes using a plastic cannula. Plasma was separated by centrifugation for aldadiene analysis.

Trained female mongrel dogs (14-19 kg)

* 3-(3-Oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone; Aldactone® is the trademark of G. D. Searle and Co. for brand of spironolactone.

TABLE I. Plasma Aldadiene Levels and Urinary Na/K Response Following Oral Administration of Spironolactone (S) and Aldadiene (A) in Adrenalectomized Rats Treated with DCA.

Treatment (total/rat)		No. of	Plasma aldadiene (log μg/100 ml × 100)					Urinary log
DCA (μg)	Spirolactone (mg)	measurements	1	2	3	4	PIA	(Na × 10)/K
—	—	11	—	—	—	—	—	1.36
12	—	7	—	—	—	—	—	.93
"	.06S	5	1.49	1.43	1.41	1.30	1.41	.87
"	.06A	5	1.41	1.38	1.32	1.23	1.34	.93
"	.3 S	5	2 24	2.04	2.01	1.89	2.04	1.00[a]
"	.3 A	5	2.45	2.13	2.00	1.86	2.11	.90[a]
"	1.5 S	5	3.18	3.16	3.04	2.84	3.06	1.19[b]
"	1.5 A	5	3.32	3.05	2.94	2.79	3.03	1.04[b]
"	7.5 S	5	4.02	4.11	4.02	3.90	4.01	1.29[c]
"	7.5 A	5	4.00	3.97	3.90	3.82	3.92	1.22[c]

Pooled s = .298 for 1, 2, 3 and 4 hr measurements of plasma aldadiene (degrees of freedom or DF = 48); least change for P of .1 = ±.32. Pooled s = .212 for plasma index of aldadiene (PIA) (DF = 16); least change for P of .1 = ±.23. Least change for P of .05 relative to DCA-treated controls = ±.10 for Na/K response; pooled s = .084 (DF = 60). [a,b,c] P = .06, .01 and .19 for difference between indicated pair of responses at .3, 1.5 and 7.5 mg doses, respectively.

Four animals/measurement for urinary Na/K; each measurement for aldadiene values was obtained from pooling plasma samples of 2 animals.

which were accustomed to procedures of bleeding from the jugular vein and bladder catheterization were used for study. Spironolactone or aldadiene as milled powder in gelatin capsules was given orally to animals injected intramuscularly in the thigh with 0.25 mg of DCA in oil, and the animals placed in metabolism cages for 6 hours. At intervals of 2 hours, a urinary specimen was obtained by catheterization of the bladder, followed by withdrawal of a blood sample (4-5 ml) from the jugular vein using a heparinized syringe. Mineralocorticoid blocking properties of the spirolactones were assessed by inhibition of the urinary Na/K response to DCA for each 2-hour period and plasma concentrations of aldadiene also determined for the corresponding periods. A detailed account of the assay procedures in dogs is found elsewhere(4).

Urinary Na and K were determined on the Beckman flame photometer, and plasma aldadiene analyzed by a fluorometric technique using the Aminco-Bowman spectrophotometer (3). Relative potencies of spironolactone and aldadiene on urinary Na/K or plasma aldadiene were computed by Irwin's method as discussed by Pugsley(9). DCA was used in conjunction with the spirolactones to induce a hyperaldosterone-like state, a condition required for demonstrating action of specific antagonists of mineralocorticoids.

Results. Table I summarizes data obtained from tests of comparable doses of the spirolactones in DCA-treated rats. Noteworthy first is the progressively higher levels of plasma aldadiene at each hourly interval after treatment with increasing doses of spironolactone or aldadiene. As the data are given in logs, a change of 0.3 unit represents a 2-fold deviation in the original values. Maximal concentrations of aldadiene were found in the first 1-2 hours post-treatment with both spirolactones, followed by a gradual decline of values in the ensuing 2 hours. Differences of response existing at 1, 2, 3 or 4 hours were all insignificant, with comparisons demonstrating P values >0.1. Accordingly, plasma index of aldadiene (PIA) determined by averaging the 4 hourly values were indistinguishable for both spironolactone and aldadiene ($P>0.1$ for differences of PIA's at the various doses). Contrastingly, there was some dissimilarity in the quantitative activity of the spirolactones as mineralocorticoid antagonists, judged by data on urinary Na/K for the total 0-4 hour period of study. This distinction of urinary effects was found at dosage levels of 0.3 to 7.5 mg, wherein spironolactone demonstrated in general stronger

TABLE II. Plasma Aldadiene Levels and Urinary Na/K Response Following Oral Dosage with Spironolactone (S) and Aldadiene (A) in Intact Dogs Treated with DCA.

Treatment (total/dog)			Plasma aldadiene (log μg/100 ml)				Urinary log (Na × 10)/K			
DCA (mg)	Spirolactone (mg)	No. of dogs	2	4	6	PIA	2	4	6	UI
—	—	9	—	—	—	—	1.18	1.41[a]	1.49[b]	1.36[c]
.25	—	22	—	—	—	—	1.06	.68[a]	.66[b]	.80[c]
"	25 S	16	1.02[d]	1.05	.87	.98	.99	.92[e]	.92[f]	.94
"	20.5A	9	.70[d]	.97	.84	.84	1.03	.58[e]	.55[f]	.72
"	50 S	9	.90[g]	1.27	1.12	1.10	1.03	1.05[h]	1.03[i]	1.04
"	40.9A	9	1.12[g]	1.08	.92	1.04	1.02	.71[h]	.67[i]	.80
"	100 S	17	1.39	1.50	1.31	1.38	1.00	1.14[j]	1.20[k]	1.11[l]
"	81.7A	10	1.35	1.39	1.22	1.32	.78	.69[j]	.71[k]	.73[l]
"	200 S	20	1.62[m]	1.68	1.56	1.62[n]	1.11	1.41[o]	1.46[p]	1.33[q]
"	163.4A	20	1.39[m]	1.56	1.43	1.46[n]	1.03	.91[o]	1.00[p]	.98[q]
"	400 S	9	1.47[r]	1.95	1.88	1.77	1.05	1.43[s]	1.72[t]	1.40[u]
"	326.8A	9	1.74[r]	1.88	1.74	1.79	.79	1.00[s]	1.21[t]	1.00[u]

Pooled s .232 and .316 for 2, 4 and 6 hr data on plasma aldadiene (DF = 60) and urinary Na/K (DF 69), respectively; pooled s = .210 for PIA (DF = 20) and .268 for urinary index of Na/K (UI) (DF = 23).

$P < .05$ for difference between responses indicated by pairs of corresponding superscript letters.

blocking manifestations than aldadiene. Reversal of the Na/K response to DCA with the spirolactones was achieved by reduction of both Na retention and K loss.

Time-response data for plasma aldadiene and urinary Na/K following treatment with equimolar doses of the compounds in dogs are illustrated in Table II.† Significant differences of plasma aldadiene randomized with respect to qualitative shifts were found in the first 2-hour period of study, but not other periods, to suggest a similar time-response pattern for spironolactone and aldadiene. PIA for the 200 mg dose of spironolactone representing the mean value at 2, 4 and 6 hours was considerably greater than that obtained with 163.4 mg of aldadiene. Apart from these data, plasma levels obtained with the spirolactones over a wide range of doses were indistinguishable. With DCA, spironolactone given at increasing doses progressively reversed the Na/K response during the 4th and 6th hour periods to levels approximating those obtained for untreated controls; similar shifts occurred in the index of urinary response (UI) or mean of the 2-, 4- and 6-hour measurements. Noteworthy in this regard are differences of response at each dosage level in the 4- and 6-hour specimens and, also, contrasting values of UI for the 2 spirolactones.

Further analysis of the data on relative abilities of the spirolactones to influence plasma aldadiene and urinary Na/K revealed interesting relationships of activities (Table III). *E.g.*, using data on PIA, spironolactone and aldadiene showed respective potencies of 1 and 0.96 in the rat study, and of 1 and 0.84 for the dog assay, to demonstrate thereby little difference from standpoint of the aldadiene response. Of related significance were differences of potencies for antimineralocorticoid properties of the spirolactones. In rats, the 0-4 hour Na/K data over dosage range of 0.7-3.5 mg showed a potency ratio of 1/0.30 for spironolactone/aldadiene ($P = 0.03$). A similar distinction of blocking effects was noted in the UI data for dogs, which described relative potencies of 1 and 0.2 for spironolactone and aldadiene, respectively ($P < 0.001$).

Discussion. An interesting observation in the present studies relates to dissociation of renal antimineralocorticoid effects and plasma aldadiene concentration in rats and dogs. Thus, while treatment with spironolactone and aldadiene orally produces aldadiene titers equivalent in magnitude or time-response relationship, spironolactone possesses a greater blocking activity than the Δ^6-metabolite. It follows from these data that renal effects of

† Molecular weight = 416.6 and 340.4 for spironolactone and aldadiene, respectively.

TABLE III. Ratios of Activities with Spironolactone (S) and Aldadiene (A) for Plasma Aldadiene and Urinary Na/K Responses in Rat and Dog Studies.

Animal	Response	Spirolactone	Relative potency	X^2 value
Rat	Plasma aldadiene	S	1	.202
		A	.96 (.79-1.17)	
	Urinary Na/K	S	1	.038
		A	.30 (.10- .93)	
Dog	Plasma aldadiene	S	1	.198
		A	.84 (.60-1.19)	
	Urinary Na/K	S	1	.533
		A	.20 (.09- .45)	

Values in parentheses = 95% limits; X^2 values > 3.841 denote invalid assays or absence of parallelism between regression lines at P of .05(9).

spironolactone are not importantly consequential to aldadiene titers in body fluids, but related to presence of spironolactone or, possibly, an intermediate(s) in its metabolic conversion to aldadiene. If major part of spironolactone administered orally exists in plasma in the form of aldadiene as noted in normal human subjects(3), the available information suggest that renal manifestations may be due to low circulating titers of a blocking agent. These laboratory data are not necessarily inconsistent with the view of clinical studies that aldadiene is a major, pharmacologically active metabolite of spironolactone(3,5,6).

In the study with spironolactone, measurements of plasma aldadiene alone may provide useful information on gastrointestinal absorption. However, in the light of present data, there will be need for caution in extending interpretation of such data to include renal manifestations, ultimately desirable as a pharmacological manifestation of spironolactone treatment. Conceivably, ratio of plasma aldadiene concentration/renal manifestation may be altered under varying conditions related to differences in pharmaceutical formulations of spironolactone, shifts in metabolic conversion, renal elimination and transport of spironolactone, or changes in physiological status, health or nutrition of the test subjects. Interpretation of data derived from both plasma aldadiene and urinary measurements can be expected to provide complementary and more reliable information on gastrointestinal absorption of spironolactone.

Summary. Relationship of renal antimineralocorticoid effects with spironolactone and plasma levels of its Δ^6-metabolite, aldadiene, was investigated in adrenalectomized rats and in intact dogs treated with DCA. In comparative tests, spironolactone and aldadiene both given orally produced equal plasma levels of aldadiene, but unequal mineralocorticoid-blocking effects. Potency ratios of approximately 3/1 and 5/1 were found for renal properties of spironolactone/aldadiene in rats and dogs, respectively. Interpretation of these data indicate that blocking activity of spironolactone is only partly accounted for by plasma titers of aldadiene.

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Received December 9, 1963. P.S.E.B.M., 1964, v115.