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REGULATION OF ALDOSTERONE IN THE GUINEA-PIG — EFFECT OF OESTRUS CYCLE, PREGNANCY AND SODIUM STATUS

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Summary. The blood concentrations of aldosterone, corticosterone and cortisol were measured in conscious, non-stressed guinea-pigs using a double isotope dilution derivative assay procedure. Aldosterone levels in the guinea-pig were high when compared with those of other species. The concentration of aldosterone, 37·9 ± 15·9 ng/100 ml ($\bar{x}$ ± SD), and cortisol, 31·8 ± 10·1 μg/100 ml, found in non-pregnant females on a moderate sodium intake was significantly greater than in males (aldosterone 22·2 ± 2·4 ng/100 ml and cortisol 19·3 ± 5·7 μg/100 ml). There was no sex difference in corticosterone concentration; females, 0·25 ± 0·06 μg/100 ml and males, 0·23 ± 0·10 μg/100 ml. The oestrus cycle had no effect on levels of the three steroids measured. Two thirds of the way through the 68-day gestation period aldosterone levels were significantly elevated compared with non-pregnant values (68·7 ± 50·9 ng/100 ml versus 37·9 ± 15·9 ng/100 ml, $p < 0·05$). Values at day 20 (33·2 ± 11·7 ng/100 ml) and day 60 of gestation (51·9 ± 21·7 ng/100 ml) were similar to those of non-pregnant animals. Cortisol and corticosterone levels were significantly elevated at 20 days gestation and they continued to rise until, at day 60, cortisol was 9 times and corticosterone 4 times higher than the non-pregnant values. Compared with a moderate Na intake, salt loading suppressed aldosterone levels and Na restriction raised them.

INTRODUCTION.

A few species have so far been shown to have elevated circulating levels of aldosterone during some part of the reproductive cycle and/or during pregnancy. Plasma aldosterone levels are increased during the luteal phase of the human menstrual cycle (Sundsfjord and Aakvaag, 1973) and its secretion varies with the phase of the oestrus cycle in the rat (Hinsull and Crocker, 1970). Pregnancy in both man and rat is accompanied by a substantial increase in aldosterone levels (Weir *et al.*, 1970; Wintour *et al.*, 1971; Whipp, Coghlan and

Wintour, 1974) and its secretion rate is increased in some dogs (Robb *et al.*, 1970). However, gestation has no effect on aldosterone levels in other species such as sheep (Wintour *et al.*, 1971) or rhesus monkeys (Wintour *et al.*, 1974).

This paper describes the influence of sex, reproductive cycle and pregnancy on the circulating levels of aldosterone in the guinea-pig.

Materials and Methods.

Mature, short-haired guinea-pigs (600-900 g) were housed either singly in metabolism cages or in groups of six per pen under conditions of natural lighting and at a room temperature of approx. 21°. Guinea-pig pellets (Mecon brand), Na content 60-100 mmol/kg and K approx. 250 mmol/kg, were available *ad libitum*. This diet provided a moderate Na intake of 2-4 mmol/animal/day and 6-10 mmol/animal/day of K. A high Na intake was achieved by replacing drinking water with 150 mmol/l NaCl affording 10-20 mmol/animal/day. The low Na diet consisted of cracked barley supplemented each day with a few green beans, and provided animals with 0·1-0·2 mmol/day of Na and 2-3 mmol/day of K. Animals were fed a particular diet for at least 20 days prior to experimentation. Vitamin C supplement was regularly added to drinking water for all diets.

Blood samples were taken between 1200 and 1300 h in the animal room by two operators who had been in regular contact with the animals. Five to eight ml of blood were collected into a heparinized syringe ($<$ 0·05 ml of 1,000 U NaCl heparin) from the conscious animal by cardiac puncture. The influence of stress was minimized by ensuring that this procedure was completed within 3 min of disturbing the animal. Three minutes is the minimum time that elapses before adrenal steroid production is stimulated by infusion of ACTH into the adrenal artery of sheep (Blair-West *et al.*, 1969) or rats (Liddle, Island and Meador, 1962).

TABLE I.

Specificity of the method for three adrenal steroids in guinea-pig whole blood.

Derivative	Number of Chromatograms	Pregnant	Non-Pregnant
		(ng/sample)	
Aldosterone diacetate	3	20·77	12·48
Aldosterone diacetate oxidized*	4†	4·81	1·58
Aldosterone diacetate oxidized	5	5·11	1·98
Aldosterone diacetate oxidized, 3-oxime	5	5·12	1·87
Aldosterone diacetate oxidized, 20β-hydroxyl	5	5·04	1·92
		(μg/sample)	
Corticosterone acetate	3†		0·174
Corticosterone acetate	4		0·174
Corticosterone acetate 3-oxime	4		0·175
Corticosterone acetate oxidized*	4		0·163
Corticosterone acetate 20β-hydroxyl	4		0·181
Cortisol acetate	2		17·42
Cortisol acetate oxidized*	3†		14·56
Cortisol acetate oxidized	4		14·45
Cortisol acetate oxidized, 3-oxime	4		14·15
Cortisol acetate oxidized, 20β-hydroxyl	4		13·50

* Chromium trioxide oxidation.

† Final chromatogram of the routine procedure.

At least 15 days elapsed between consecutive blood sampling from the same animal. The whole blood samples for adrenal steroid measurement were stored at −20°.

Blood samples were analysed for aldosterone, corticosterone and cortisol using the double isotope derivative dilution procedure of Coghlan and Scoggins (1967). Specificity of the method was established for aldosterone in samples from pregnant and non-pregnant animals and for corticosterone and cortisol in a sample from a non-pregnant animal by multiple derivative formation and further chromatography using methods as described in the procedure above (Table 1).

The pH of blood stored in a sealed syringe was determined within 15 min of collection and Na and K concentrations in plasma and urine were measured using a Technicon Auto Analyser.

All results are expressed as mean ± SD and statistical significance was obtained using students *t*-test.

RESULTS.

Blood versus plasma steroid concentration.

The concentrations of aldosterone, corticosterone and cortisol in the blood of a female guinea-pig, 38·0 ng/100 ml, 0·35 and 81·6 μg/100 ml respectively, were lower than in plasma 45·0 ng/100 ml, 0·38 and 114·0 μg/100 ml.

Sodium intake. In 7 of the 8 guinea-pigs studied a high salt intake suppressed aldosterone levels compared with a moderate sodium intake, while a low sodium diet consistently increased circulating levels (Table 2). Dietary sodium intake had no effect on blood cortisol or corticosterone levels.

TABLE 2.

Effect of sodium intake on blood aldosterone concentration (ng/100 ml) in guinea-pigs.

GP	Sex	High Na intake	Moderate Na intake	Low Na intake
13	M	3·5	9·3	82·8
14	M	11·8	42·6	87·2
15	M	12·5	10·2	669·2
25	F	16·0	34·1	160·9
50	F	15·9	16·2	54·8
22	F	3·2	18·8	—
24	F	14·7	51·3	—
56	F	14·6	53·3	—

Range of daily urinary output of sodium and potassium.

Na (mmol)	7–30	1–6	Not detectable–0·4
K (mmol)	4–15	4–15	1–4

The output of sodium and potassium in urine fluctuated from day to day as did the food intake but was usually within the ranges shown in Table 2. The transition to a low sodium diet was usually accompanied by reduced food intake and weight loss for the first 3-4 days, but by the time blood samples were collected the body weight was stable or increasing.

Sex. Male and non-pregnant female guinea-pigs fed the moderate diet had different values for a number of blood parameters (Table 3). Male guinea-pigs exhibited significantly higher plasma [Na] and [K], haematocrit and blood pH and lower aldosterone and cortisol concentrations than did females. Corticosterone concentrations were similar in both sexes.

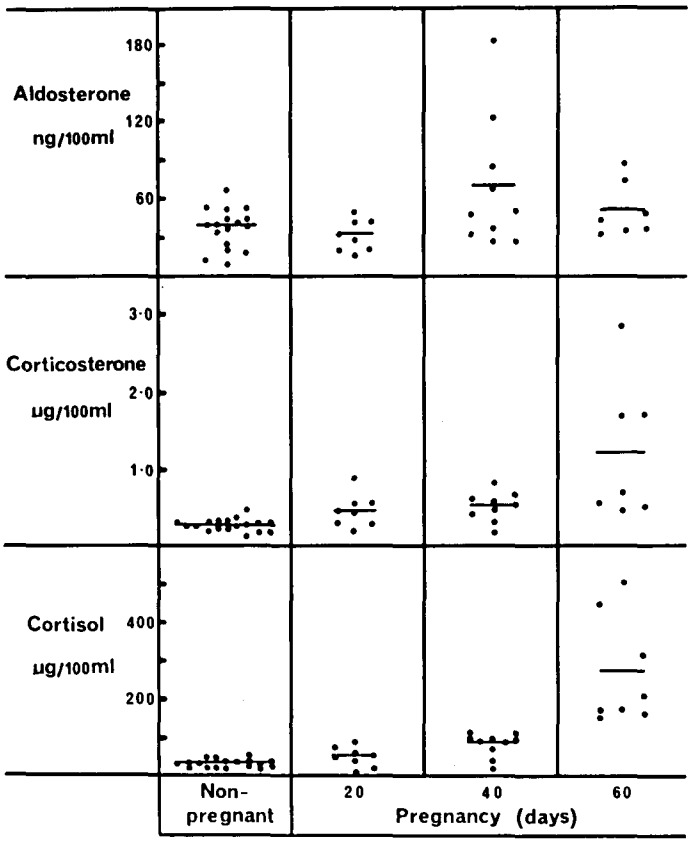

Fig. 1. Peripheral blood steroid concentrations in non-pregnant and pregnant guinea-pigs. The bars represent the mean for each group.

Oestrus cycle. There was no significant difference ($p > 0{\cdot}05$, students *t*-test for paired observations) in the steroid levels at diestrus compared with those at oestrus, in the next cycle, for the 7 animals studied. Mean concentration for aldosterone at diestrus was $34{\cdot}2 \pm 21{\cdot}3$ ng/100 ml and at oestrus $27{\cdot}3 \pm 18{\cdot}2$ ng/100 ml ($n = 7$). Between animal variability, particularly for aldosterone concentration, was large in this and other experimental groups. The within animal variation was much less.

Pregnancy with moderate sodium intake. Aldosterone levels were elevated only at day 40 of pregnancy ($p < 0{\cdot}05$) (Fig. 1). Gestation in the guinea-pig lasts for 68 days. The mean of a group of 10 pregnant animals, at day 40, was

68·7 ± 50·9 ng/100 ml which is less than double that of the non-pregnant group, 37·9 ± 15·9 ng/100 ml (n = 19). At 20 and 60 days gestation, blood aldosterone levels were similar to those of non-pregnant animals, being 33·2 ± 11·7 (n = 8) and 51·9 ± 21·7 (n = 8) ng/100 ml respectively. Sequential sampling from 5 animals before, during and after pregnancy did not conclusively establish an effect of pregnancy on blood aldosterone levels (Fig. 2). There was a definite rise only for 2 animals.

TABLE 3.

Comparison of sex differences in blood pH, haematocrit, plasma [Na] and [K] and peripheral blood steroid concentrations in conscious, non-stressed guinea-pigs (mean ± S.D. (n)).

	Males	Females	
Blood pH	7·47±0·04 (14)	7·41±0·05 (33)	$p<0·001$
Haematocrit (%)	41·1±5·1 (45)	37·4±6·1 (34)	$p<0·01$
Plasma [Na] (mmol/l)	144±3 (40)	139±3 (41)	$p<0·001$
Plasma [K] (mmol/l)	5·5±0·5 (40)	5·0±0·5 (41)	$p<0·001$
Aldosterone (ng/100 ml)	22·2±12·4 (10)	37·9±15·9 (17)	$p<0·05$
Corticosterone (μg/100 ml)	0·23±0·10 (10)	0·25±0·06 (19)	NS
Cortisol (μg/100 ml)	19·3±5·7 (10)	31·8±10·1 (19)	$p<0·01$

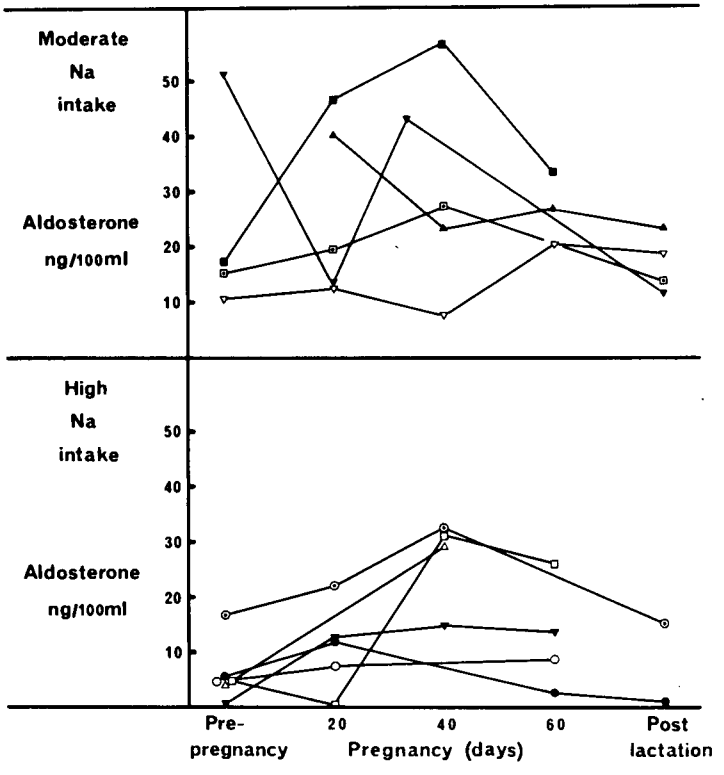

Fig. 2. Sequential blood aldosterone levels in guinea-pigs before, during and after pregnancy: effect of moderate or high Na intake.

Pregnancy with high salt intake. Sodium loading caused an overall suppression of aldosterone concentration (Fig. 2). However, the aldosterone levels in 4 of the 6 animals studied were substantially increased during pregnancy.

Corticosterone and cortisol concentration were both significantly elevated when examined during early pregnancy, $p < 0{\cdot}001$ and $p < 0{\cdot}01$ respectively. The levels of these steroids increased further during mid-gestation and very high values were observed just before term (Fig. 1).

DISCUSSION.

The blood levels of aldosterone for guinea-pigs with a moderate sodium intake are quite high compared with those found in other species when the same method of analysis was used. One can compare $37{\cdot}9 \pm 15{\cdot}9$ ng/100 ml blood for guinea-pigs with values in man of $5{\cdot}8 \pm 4{\cdot}3$ ng/100 ml plasma (Coghlan and Scoggins, 1967), rhesus monkey $25{\cdot}9 \pm 7{\cdot}3$ ng/100 ml plasma (Wintour *et al.*, 1974), sheep $2{\cdot}1 \pm 1{\cdot}7$ ng/100 ml blood (Scoggins *et al.*, 1974), dog $2{\cdot}1 \pm 3{\cdot}6$ ng/100 ml blood, fox $13{\cdot}9 \pm 3{\cdot}2$ ng/100 ml blood, cow $2{\cdot}9 \pm 2{\cdot}5$ ng/100 ml blood (Oddie *et al.*, 1975) and rat $12{\cdot}9 \pm 8{\cdot}9$ ng/100 ml (Whipp *et al.*, 1974). Even under conditions of considerable sodium intake, levels in the guinea-pig were appreciable (Table 2). The report by Underwood and Dodeja (1974) on the secretion rate of aldosterone supports the finding of relatively high circulating levels in this species. They found that, on a body weight basis, the aldosterone secretion rate is approx. three times that found for man.

Malinowska and Nathanielsz (1974) found a mean plasma aldosterone concentration of 7·2 ng/100 ml in four conscious, non-stressed, adult, male guinea-pigs. In the present study (Table 2), sodium loading produced levels comparable with those in their report. Since no electrolyte intake data was provided, it is possible that their levels reflect a high level of sodium intake.

Some other reports on cortisol levels in conscious, non-stressed guinea-pigs have given values lower than those in this study. Blood cortisol concentrations in the present study were 19·3 μg/100 ml for males and 31·8 μg/100 ml for females compared with Malinowska and Nathanielsz's (1974) value for male plasma of 8·4 μg/100 ml and that of Fazekas, Homoki and Teller (1974) of 7·5 μg/100 ml plasma for males and 12·6 μg/100 ml plasma for females. Speculative reasons for this discrepancy in cortisol values could include genetic differences in corticosteroid synthesis or metabolism, as has been demonstrated in guinea-pigs by Burstein, Kimbal and Bhavnani (1963) or strain differences in plasma corticosteroid-binding capacity as has been mentioned in the review by Shire (1974).

The possibility that measurement of adrenal steroids in blood rather than plasma might give high values due to concentration of steroids in the red cell fraction was unlikely, since plasma concentrations of the sample examined were higher than those in whole blood.

This study indicates that the concentrations of all three adrenal steroids in the guinea-pig are similar at oestrus and diestrus. The data of Diamond, Rust and Westphal (1969), which suggested that plasma cortisol in the guinea-pig peaked at oestrus and was low at mid-diestrus, was complicated by ether stress and the analysis of pooled samples.

Pregnancy had a minimal effect on the aldosterone levels of guinea-pigs. Perhaps, like the dogs in the study by Robb *et al.* (1970), only some guinea-pigs have elevated levels during pregnancy, as suggested by Fig. 2. If this is the case, the results of the experiment involving high sodium intake during pregnancy indicate that the stimulus which elevates aldosterone levels is not eliminated by sodium loading.

The very large rise in corticosterone and cortisol concentrations during gestation in the guinea-pig are most likely due to the large increase in corticosteroid-binding protein which begins at about day 20 (Diamond *et al.*, 1969).

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