Superfoetation in CBA Mice

Superfoetation is defined[1] as the occurence in the same animal of 2 pregnancies at different stages of development. Such anomalous gestations are generally considered[1-3] to be the result of a further ovulation and fertilization occuring after the beginning of pregnancy. Apparent spontaneous superfoetation is not very common, although several cases have been reported in cat[4], in sheep[5,6], in pig[6,7], in rat[8,9] and in man[10]. Some examples have been described in mice by CREW and MIRSKAIA[11], LITTLEFORD and GYSIN[12] and more recently by BARNETT and MUNRO[2] in mice of a mixed stock derived from 4 inbred strains, A, A2G, C57B1 and GFF.

Résumé. Plusieurs cas de superfoetation ont été observés chez des souris de race CBA. Compte tenu des conditions expérimentales, il apparait que ces cas de gestation anormale sont dus soit à la fécondation d'ovules produits

Analysis of anomalous gestations

Mouse No.	First litter after mating				'Fatherless' litter			
	Days since last mating	No. weaned			Days since last mating	No. weaned		
		Total	♂	♀		Total	♂	♀
1	24	3	0	3	45	6	4	2
	22	5	4	1	51	2	1	1
2	35	5	3	2	58	1	1	0
3[a]	27	4	2	2	47	4	2	2

[a] Offspring from female No. 1.

Similar observations have been recorded in our laboratory in a small breeding colony of an inbred strain. 60 females from the CBA strain, 11–13 weeks old, were mated, 1 male with 3 females, with animals from the same strain. Pregnant females were removed 5 to 10 days before parturition and were kept in separate cages until 30 days after weaning of the first litter. As shown in the Table, 2 females which were removed from the male gave a second litter 21 and 23 days respectively after the first delivery. Both sexes were represented in each litter. Such anomalous gestation occurred again with the first female and also in 1 pair sired by the same animal.

The fact that the females were removed from the males at least 5 days before parturition excludes the possibility of insemination during late pregnancy. As BARNETT and MUNRO[2] have pointed out, parthenogenesis or storage of spermatozoa in the females are unlikely to occur. Thus, such anomalous gestations must be either the result of delayed implantation upto parturition of the first pregnancy, of blastocyts in females which have ovulated and mated after the beginning of the first pregnancy or of delayed implantation of blastocytes from the same ovulation and fertilization which also gave rise to the first pregnancy. An argument in favour of the second alternative may be seen in the fact that the litter size of both pregnancies together approaches that normally found in a single pregnancy. In addition, it is of interest to note that the strain studied has apparently a tendency for a delayed implantation as seen from the somewhat longer pregnancy recorded in some cases (Table). Since the same phenomenon was observed in the offspring of 1 female showing superfoetation, it is possible that this character can, to some extent, be inherited[13].

peu de temps après le début de la gestation, soit à un retard dans l'implantation d'un certain nombre d'ovules qui ont donné la première nichée. Nos observations sembleraient montrer que ce phénomène pourrait être plus fréquent que ce que l'on avait cru jusqu'à présent.

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Activation of Aldosterone and Renin Secretion by Thermal Stress

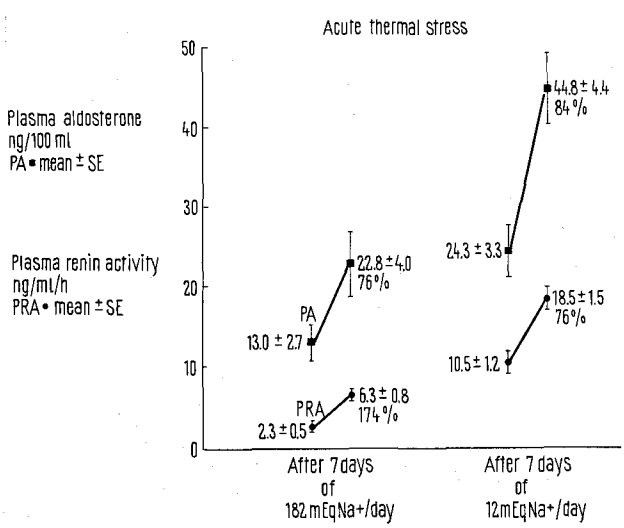
Thermal stress with or without exercise, or thermal stress combined with sodium deprivation, increases mineralocorticoid excretion in man[1-5]. A sodium deficit seems necessary to increase aldosterone production during heat exposure since urinary tetrahydroaldosterone excretion rates increased during acclimatization to heat when sodium deficits of 140–320 mEq were incurred, but not during acclimatization with replacement of sodium losses[6]. However, no data exist on plasma aldosterone (PA) concentrations and plasma renin activity (PRA) in men exposed to acute thermal stress during normal sodium intake and sodium deprivation.

In our study, 12 health young male volunteers, ages 20 to 30, were maintained under controlled metabolic conditions during cool weather. Daily activities were unrestricted except vigorous physical activity was not allowed. Each subject was given a high sodium diet (> 300 mEq daily) during 1st week; a 'normal' sodium and potassium diet (182 mEq Na, 68–72 mEq K daily) during the 2nd week; and a low sodium, 'normal' potassium diet (12 mEq Na, 65–73 mEq K daily) during the 3rd week. At the end of the 'normal' and low sodium diet periods venous blood was obtained from each subject between 07.00 and 08.00 h after 30 min of sitting quietly. Subjects were then thermally stressed for 1 h in an environmental chamber (46–51°C, approximately 90% RH). After leaving the chamber, the subjects again sat quietly for 30 min and venous blood was again collected.

For PRA, blood was collected in a chilled tube containing 1.25 mg ethylenediaminetetraacetic acid, disodium salt, per ml blood, mixed, centrifuged at 4 °C and plasma frozen at –20 °C until analysis. PRA was estimated by radioimmunoassay of angiotensin-I (minor modification of the method of HABER et al.[7]) employing an antibody against angiotensin-I rabbit serum albumin and ^{125}I -angiotensin-I as a tracer. Angiotensin generation was estimated from 1-, 2- and 3-hour incubations of triplicate samples at 37 °C, pH 6 without the addition of exogenous renin and the results expressed as ng angiotensin-I generated/ml plasma/h.

For PA concentration, blood was collected in a chilled, heparin-coated tube and centrifuged at 4 °C. 20 ml of plasma was mixed with 1.9 ng (577 dpm) 4-^{14}C-aldosterone (53 mc/mmole); 0.5 ml 1.25 N NaOH was added and the plasma extracted twice with 60 ml ice-cold redistilled methylene dichloride. After neutralization of the extract with 6 ml 0.1 *M* acetic acid followed by 3 washes with 3 ml glass-distilled H_2O. aldosterone was estimated by a double isotope derivative method using ^{3}H-acetic anhydride (1000 mc/mmole) as the labeling reagent[8]. For purification of the derivatives paper chromatography[8,9] was used in the following order: I. cyclohexane:benzene: methanol:water (100:40:100:20); II. cyclohexane:benzene:methanol:water (100:70:100:25); III. system I again; IV. mesitylene:methanol:water (120:80:40). Labeled aldosterone monoacetate was purified in systems I and II and the diacetate in systems III and IV. Overall recovery of the labeled indicator (13 estimations) was 15.7 ± 5.2 (S.D.) ± 1.4 (S.E.) percent. The nonspecific blank of the method, estimated by processing 20 ml distilled water, was – 0.36 ng ± 0.639 (S.D.) ± 0.133 (S.E.) per sample. Values obtained on pooled plasma with 2 replications agreed within ± 8% (n = 5).

After thermal stress at the end of the 'normal' sodium diet period, mean PRA increased from 2.3 to 6.3 ng/ml/h (174%, $p < 0.01$) and mean PA increased from 13.0 to 22.8 ng/100 ml (76%, $p < 0.01$) (Figure). After 1 week of sodium deprivation mean PRA increased from the initial control value of 2.3 to 10.5 ng/ml/h (357%) $p<0.01$) and mean PA increased from the initial control value of 13.0 to 24.3 ng/100 ml (87%, $p < 0.05$). Acute thermal stress further increased mean PRA from 10.5 to 18.5 ng/ml/h (76%, $p < 0.01$) and mean PA from 24.3 to 44.8 ng/100 ml (84%, $p < 0.05$). This stress did not produce a significant change in packed cell volume. Mean packed cell volumes before and after thermal stress were 44.33 ± 0.60 (S.E.) and 44.75 ± 0.77, respectively, during 'normal' sodium intake, and 45.73 ± 0.71 and 45.80 ± 0.57, respectively, during the low sodium intake. These results confirm previous observations that sodium deprivation stimulates both renin and aldosterone secretion and indicate that high thermal stress, per se, is a potent stimulator of renin and aldosterone secretion both in the presence and absence of sodium deprivation[10]. Consequently, thermal stress can be used to test the integrity of these hormone production systems.



Influence of acute thermal stress on plasma aldosterone concentration and plasma renin activity after normal and low Na^+ diets in 12 healthy male subjects.

Résumé. Un stress thermique aigu est un activateur rapide et puissant de la sécrétion du système rénine-angiotensine et de l'aldosterone au cours d'une alimentation normo ou hyposodée, et peut être utilisé pour mesurer l'intégrité des systèmes de production de ces hormones.

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