

changes in thyroid morphology were present but less extensive 8 days after tumor inoculation.

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Adrenocortical and Gonadal Responses of Female Mice to Increased Population Density. (25825)

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Increased adrenal cortical and decreased gonadal function result from grouping male mice, the magnitude of these responses depending on population density(1). Similar gonadal and adrenocortical changes occur in female mice from populations of mixed sex (2,3,4,5). The changes in male mice are related to intraspecific strife, their magnitude in an individual to its position in a social hierarchy(6). It is not known if the adrenals of female mice and rats react similarly when in groups of females only, although it is known that reproduction is adversely affected with suppression of estrus(7,8). This paper explores the effects on adrenal glands and gonadal function of placing females together in groups in absence of males.

Methods. In each of 3 experiments female mice were placed in individual cages at 12-13 g. Four weeks later the mice were randomized and assigned according to groups or to remain isolated. Ten days after grouping, the mice were sacrificed and weights of adrenals, thymus, spleen, ovaries, uterus and, in Exp. 3, the preputials obtained after fixation in 10% neutral formalin(2,9,10). Adrenals were

serially sectioned at 6 μ in all experiments, and ovaries, uterus, and vagina were sectioned in Exp. 3. Widths of adrenal X-zones, zonae fasciculata-reticularis, and number of cells in straight cords of the latter were determined histologically(2). Eleven groups of 6 each of nulliparous females of the albino NMRI strain were used in Exp. 1. Seven groups of 6 parous mice each, of the same strain, were used in Exp. 2. Parous mice were used to avoid confounding the adrenal weight data by the X-zone, which is involuted at first pregnancy in females. For this experiment male and female littermates were placed, one pair to a cage, at weaning. Males were removed when the females were obviously pregnant for the first time, at 10 or 11 weeks of age. The young were removed at birth and the females isolated until the required number were obtained. Seven groups of 4 each of inbred, nulliparous females, of a strain originally derived from wild mice, were used in Exp. 3. This strain is much more aggressive and more reactive to grouping than laboratory albino mice(9). It is known that females of this strain are in estrus a high percentage of the time and do not undergo spontaneous pseudo-pregnancy.

Results. The results are summarized in Table I as mean organ weight and its stand-

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TABLE I. Organ Weights Expressed as Means and Standard Errors of Nulliparous Albino (Exp. 1), Parous Albino (Exp. 2), and Nulliparous Wild Stock (Exp. 3) Female Mice, in Groups Consisting of Females Only, and with Their Isolated Controls. In grouped mice, the means are means and standard errors of group means.

Exp.	No.	Mean body wt change	Adrenals	Width X-zone	Width fasc.-ret.	Cells fasc.	Thymus	Spleen	Ovaries	Uterus	Preputials
1	Isolated	12	9.70 $\pm$.78	15.9 $\pm$ 2.9	16.5 $\pm$.5	15.6 $\pm$.6	74.6 $\pm$ 5.1	107.5 $\pm$ 6.7	10.2 $\pm$.8	104.7 $\pm$ 14.6	
	Grouped	11	10.1 $\pm$.41	11.1 $\pm$.8	20.3 $\pm$.3	21.4 $\pm$.7	87.0 $\pm$ 2.4	117.4 $\pm$ 5.3	11.3 $\pm$.4	94.5 $\pm$ 19.3	
	p		.70	.02	.0001	.0001	.06	.50	.70	.70	
2	Isolated	16	7.83 $\pm$.38	5.5 $\pm$ 1.3	20.8 $\pm$.8	19.0 $\pm$.7	54.9 $\pm$ 3.9	127.7 $\pm$ 5.4	16.1 $\pm$ 1.1	158.8 $\pm$ 14.1	
	Grouped	7	7.60 $\pm$.30	2.8 $\pm$.6	23.8 $\pm$.6	21.6 $\pm$.8	60.4 $\pm$ 10.3	145.9 $\pm$ 7.2	17.2 $\pm$ 2.9	177.1 $\pm$ 6.7	
	p		.70	.10	.01	.05	.70	.06	.50	.30	
3	Isolated	7	12.08 $\pm$.53	17.1 $\pm$ 2.0	17.5 $\pm$.5	16.9 $\pm$.6	74.3 $\pm$ 6.1	86.3 $\pm$ 9.6	7.71 $\pm$.69	156.7 $\pm$ 15.8	13.1 $\pm$ 2.5
	Grouped	7	11.66 $\pm$.31	17.6 $\pm$.8	21.1 $\pm$.8	18.9 $\pm$.4	65.5 $\pm$ 4.9	99.8 $\pm$ 8.5	9.14 $\pm$.40	75.1 $\pm$ 4.1	15.1 $\pm$ 2.0
	p		.50	.90	.01	.02	.30	.40	.10	.001	.30

ard error. Only mean values for each group are used for purposes of statistical evaluation, to avoid within-cage effects and to test reliably the effects of grouping. Consequently, mean weights given for the grouped animals are means of the group means.

There is no significant decline in X-zone width in the grouped mice in Exp. 3. However, histologically the X-zones of grouped mice showed marked involutional changes not seen in their isolated controls. Also, uterine and vaginal sections from this experiment indicated that most of the grouped mice were in anestrus or diestrus at sacrifice, whereas 5 of the 7 isolated controls were in estrus. Uterine weight appeared closely related to stage of the estrous cycle. Lower uterine weight in grouped mice reflects the fact that most were diestrus or anestrus, conversely with the isolated controls. If the stages of estrus are assigned values as follows: anestrus 0, diestrus 1, metestrus 2, proestrus 3, and estrus 4, the grouped mice have a mean "estrus value" of 1.27 ± 0.24 and the isolated mice 3.29 ± 0.47 , the difference being highly significant ($P < 0.01$).

Discussion. Grouping appears to result in an increase of adrenocortical activity in female mice, which is not reflected by adrenal weight since mild hyperplasia of the adrenal fasciculata-reticularis in grouped females was accompanied by an equivalently increased involution of the X-zone. Combined widths of the fasciculata-reticularis and X-zones remained constant. The X-zone is involuted by weak androgens(9,11), some of which are known to be secreted by the adrenal cortex (12-15) and to be stimulated by ACTH(13-16). Increased involution of the X-zone of grouped mice may have been caused by increased adrenal androgens. Greater mean preputial weight in grouped mice (Exp. 3), although not significant, is consistent with this interpretation and increased ACTH is indicated by hyperplasia of the fasciculata-reticularis. The marked adrenocortical response in female rats and mice from populations of mixed sex must largely be due to the presence of males, increased female aggressiveness during pregnancy and lactation, or both.

Splenic weight in male mice increases with grouping(17,18). Mean splenic weight of grouped females, in every experiment, was greater than in isolated mice, although the differences were not significant. It seems logical to conclude that grouping induces an increase in splenic size in female mice.

Ovarian weight was not affected by grouping. Uterine weight was greater in isolated than in grouped nulliparous wild stock and albino nulliparous female mice, but not significantly in the latter. However, since the changes in the albinos were in the same direction as the significant change in wild stock mice, it appears that a diminution in uterine size is associated with grouping, which is consistent with the results of Andervont(7) and of Whitten(8), showing inhibition of reproductive function in grouped female mice. The fact that decreased uterine weight correlated with diestrus and anestrus in the wild stock mice and that this strain is known not to undergo spontaneous pseudopregnancy lends weight to the conclusions. Isolated wild stock females are in estrus a major portion of the time, so that a large proportion in diestrus, with infantile uterine weights, suggests that these mice were anestrus, corresponding to Whitten's(8) results.

Summary. Nulliparous and parous albino, and nulliparous wild stock female mice were subjected to grouping. Weights of adrenals, thymus, spleen, ovaries, and uterus were obtained. Widths of the adrenocortical X- and fasciculata-reticularis zones were measured and number of cells in columns of fasciculata-reticularis determined. Weights of adrenals, thymus, and ovaries were not significantly affected by grouping. Increased adrenocortical activity with grouping was indicated by cellular hyperplasia of the zonae fasciculata-

reticularis. Increased involution of the adrenal X-zone with grouping may be accounted for by increased adrenal androgens. Diminished uterine weight in grouped nulliparous females suggests suppression of gonadal function and gonadotrophin secretion. Based partly on prior evidence, it was concluded that an increase in splenic weight was associated with grouping. The observed responses account only in small part for the degree of adrenocortical activity and reproductive suppression in populations of mixed sex.

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