The Modification of the Teratogenic Action of Cortisone by Parity[1]

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During an investigation of the genetics of strain differences in the response of mice to the teratogenic effects of cortisone (*1, 2*), it was observed that the frequency of cleft palate in the offspring of treated mice varied with the parity of the female. The cross which permitted the most precise statistical analysis of this phenomenon was the backcross of F_1 females from reciprocal crosses between strains C57BL/6Jax and A/Jax (*3*) to strain A/Jax males. In this cross the F_1 females had comparatively large litters, many litters, and a mean frequency of the anomaly intermediate between those of the parent strains (*2*).

TABLE 2

TEST OF SIGNIFICANCE BETWEEN FIRST AND ALL OTHER LITTERS FROM TREATMENTS BEGUN ON GESTATION DAYS 10 TO 12

	Parity		Total
	1	2–9	
Normal	85	361	446
Cleft palate	39	73	112
Total	124	434	558
	$\chi^2 = 11.8$, $P < 0.001$		

TABLE 1

NUMBER AND PER CENT AFFECTED OFFSPRING ACCORDING TO PARITY RESULTING FROM TREATMENTS BEGUN ON GESTATION DAYS 10 TO 12

	Parity							Total
	1	2	3	4	5	6	7–9	
Total number of offspring	124	123	94	78	63	39	37	558
Number with cleft palate	39	26	19	13	8	5	2	112
Percentage with cleft palate	31.5	21.1	20.2	16.7	12.7	12.8	5.4	20.1

Animals were fed Purina Fox Chow and water ad libitum. Pregnant females were treated intramuscularly in the flank with 2.5 mg of cortisone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate)[2] on 4 successive days of the gestation period. None of 87 offspring of untreated F_1 females had a cleft palate.

Pregnant females were treated beginning on the 6th to 17th days of gestation. The highest incidences of the defect were caused by treatments which began on gestation days 10, 11, and 12. The results for these days were pooled, and a statistical test was made on this material to determine whether there was any effect of parity on the frequency of cleft palate. The results show that with each successive litter the frequency of the defect decreased (Table 1); however, only the frequency of the defect in 1st litters (32%) is significantly greater, statistically, than the frequency in all other litters (16%, Table 2), or any other litter.

The possibility was considered that those females most susceptible to the teratogenic effects of cortisone also became sterile or died sooner than the less susceptible females, and that their progressive elimination from the population might have caused the decreasing incidence of the defect in successive litters. Thirty-three females were involved in this experiment. The modal life span of these animals (i.e., age at death or discarding) was 13 months. Of these 33 females, 19 lived less than 13 months (total of 151 months, mean = 7.95 months) and had 84 conceptions (mean = 4.42 conceptions). The remaining 14 lived a total of 186 months (mean = 13.29 months) and had 104 conceptions (mean = 7.43 conceptions). The ratio of number of conceptions to life span in months, however, is the same, 0.56, for females that lived less than 13 months and for females that lived 13 or more months ($t = 0.08$, d.f. = 31, $P > 0.9$). It is apparent, therefore, that these 2 groups of females were equally fertile.

Ten of the 33 females conceived less than 5 times (total 25, mean = 2.50 conceptions); these lived a total of 70 months (mean = 7.0 months). The other 23 females conceived a total of 163 times (mean = 7.09 conceptions) and lived a total of 267 months (mean = 11.61 months). The ratio of number of conceptions to life span in months for the 1st group is 0.36, for the 2nd group, 0.61. The conception rate, therefore, is

TABLE 3

FRACTION OF AFFECTED OFFSPRING IN FIRST FOUR LITTERS OF FEMALES WITH LESS THAN FIVE AND FIVE OR MORE CONCEPTIONS, RESULTING FROM TREATMENTS BEGUN ON GESTATION DAYS 10 TO 12

Times females conceived	Parity			
	1	2	3	4
Less than 5	11/29	5/38	3/11	0/ 6
5 or more	28/95	21/85	16/83	13/72
χ^2	0.40	1.47	0.05	—
P	0.53	0.22	0.82	0.32*

* Probability derived by use of Fisher's (*4*) exact treatment of the 2 × 2 table.

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[2] Cortisone generously supplied by J. H. Laurie, Merck and Co., Ltd., Montreal.

almost twice as great for the group with 5 or more conceptions than for the group with less than 5 conceptions. The difference, however, is not statistically significant ($t = 1.12$, d.f. $= 31$, $P = 0.2$–0.3).

It was thought advisable, nevertheless, to test the frequencies of the defect in the offspring of the latter 2 groups of females against each other. The results show (Table 3) that each of the first 4 litters of the "sterile" females is not statistically different from its counterpart from the "nonsterile" females. The possibility is excluded, therefore, that the decreasing frequency of the defect in successive litters was due to the sterilization of the more susceptible females, and the conclusion can be drawn, from Table 1, that primigravid females are significantly more susceptible to the teratogenic effects of cortisone than multigravid females.

It is interesting to note (Table 3), however, that a good deal of the parity effect is due to the "sterile" group, and that when the results for this group are not considered, the difference in incidence of the defect between the 1st and 2nd litters of the "nonsterile" group is not significant ($\chi^2 = 0.29$, $P = 0.59$).

References

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Effects of Oral Administration of Spanish Moss, *Tillandsia usneoides* L.

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Spanish moss, *Tillandsia usneoides* L., is described by Small (*1*) as a picturesque and characteristic feature of the southern coastal states. The dried processed inner fiber of the strand of moss has long been utilized in the upholstery industry.

In conjunction with our studies on the estrogenic substance in Spanish moss (*2*), a feeding experiment was devised to show the effects of oral administration of the ground moss to young and adult albino rats.

Twenty fertile adult albino rats of approximately the same age and weight, divided equally as to sex, were placed in two groups each containing 5 males and 5 females. The males and females of each group were separated for 30 days, during which time group I was fed a commercial ration of dog cubes, and group II the same ration powdered and mixed with an equal part by weight of ground Spanish moss.

After feeding this diet 30 days no significant differences in weight were observed.

The rats were then paired with those of opposite sex of their same group and the diets continued. On the 23rd day after the mating, a litter was born to 1 female in each group; 12 young in the litter of group I and 5 in the litter of group II. There was no significant difference in weight at birth. When 18 days old, the young of group I averaged 19.5 g, and those of group II 6.5 g. All 5 young of group II died before they were 20 days old, and only this one litter was born to this group. The animals in group I reproduced normally.

Estrogen administration begun before 4–6 weeks of age inhibits growth and development, but if begun after full growth is attained it does not cause a loss of weight in rats (*3*). The administration of estrogens to lactating mothers inhibits growth of young (*4*). Zondek (*5*) showed a direct relationship between growth inhibition and quantity of hormone administered. The skeletal development as well as organ size is affected. The action is explained by an inhibitory mechanism of the hormone on the anterior pituitary.

Hormonal castration of cockerels by stilbestrol implants is frequently utilized to improve meat quality and stimulate growth. Estrogens apparently have no activity on quantity or quality of meat produced from swine (*6*). A recent publication from Purdue University (*7*) shows that 60 mg-stilbestrol implants in yearling steers will increase daily weight gain by 10%; and 120 mg-implants by 18%. However, 180 mg-implants of testosterone will depress daily weight gain by 2.6%. It is significant that less food is required for a gain of 100 lbs of body weight by animals which received stilbestrol; 4% less food concentrate being required by those receiving 60 mg-implants; and 10% less food by those receiving 120 mg-implants, while the testosterone-treated steers required more food.

In view of the recent trend to improve meat quality by administration of estrogens, it may be possible to utilize the waste material from the processing of fibers of Spanish moss for the upholstery industry, as a fodder supplement for beef cattle. The high fiber content of this waste material would make it unsuitable for feeding swine, but cattle, sheep, and goats could digest the fibers and utilize in addition the vitamin, mineral, and carbohydrate constituents. Webber, *et al.* (*8*) showed the presence of an antibacterial substance in moss. Halligan gives the analysis of green moss (*9*) as:

Protein	3.68%	Iron and aluminum oxide	0.28%
Carbohydrate	15.9%	Phosphate	0.032%
Fiber	8.24%	Calcium oxide	0.058%
Water	69.5%	Sodium oxide	0.58%
Ash	1.57%	Potassium oxide	0.31%

The Florida Agricultural Experiment Station showed that Spanish moss contains more food value than oat straw (*10*) and that moss contains 1.5 mg% of β-carotene, the precursor of vitamin A (*11*). Since the estrogen of Spanish moss is effective by oral administration, it may be possible to utilize the waste from the processing of fibers for the upholstery in-