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Growth Rates: Influence of Prednisolone, Sex and Crowding in Rats

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GROWTH RATES: INFLUENCE OF PREDNISOLONE, 
SEX AND CROWDING IN RATS

by

Karen M. Allen

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Master of Arts
Department of Psychology

Western Michigan University
Kalamazoo, Michigan
August 1983
ACKNOWLEDGEMENTS

I would like to thank members of the Laboratory in the Behavioral Effects of Cancer Therapy for their help and the use of animals and facilities of that program. Also appreciated was the statistical assistance provided by Jeff Gallus and the help of Andrew Arneson in the exhausting chore of 16 days of injections of 72 animals. Special thanks are given to Robert Sewell for unflagging and invaluable editorial assistance. Support from Dr. Malcolm Robertson, my thesis advisor, and from Drs. Chris Koronakos and Neil Kent was greatly appreciated. This research was supported in part by a Graduate Student Research Award from the Graduate College of Western Michigan University and in part by Department of Psychology assistance to the laboratory.

Karen M. Allen
GROWTH RATES: INFLUENCE OF PREDNISOLONE, SEX AND CROWDING IN RATS

Karen M. Allen, M.A.
Western Michigan University, 1983

Growth inhibition is frequently noted in the chronically ill child. Causes of such growth rate decrements appear multiple and include both internal and environmental factors. The present study analyzed growth of rats by manipulation of glucocorticoid treatment, housing conditions and sex of subject in a search for potential interactions. Specifically, body weights were assessed daily in post-weanling male and female Sprague-Dawley rats which received 16 daily I.P. injections of prednisolone acetate (0.0, 2.0, or 4.0 mg/kg) under either individual or group-housed (6/cage) conditions. Effects of dose and of sex were found, while a main effect of housing was lacking. Interactions of dose X housing, as well as dose X sex, were demonstrated. Results are discussed in terms of alterations in protein synthesis, bone growth, and growth hormone as influenced by glucocorticoids. Also discussed are stress and housing effects on growth, as well as possible mechanisms for the noted sex X housing X dose interaction.
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CHAPTER 1

INTRODUCTION

Decreased growth rates have been observed in chronically ill children receiving glucocorticoid and other treatments (e.g., Cheek, 1968; Falliers, Tan, Szentivanyi, Jorgensen, and Dukantz, 1963). Often the cause of such decrements has remained obscure, perhaps due in part to growth's multiple determination. Both intrinsic and extrinsic influences can be simultaneously operative, and may interact to change growth rates. One major set of factors modulating growth is organismic variables. Age-related factors, for instance, are important determinants (Cheek, Migeon, and Mellits, 1968). Gender and associated hormonal status are well-known growth modulators. For example, maturational changes such as skeletal proportion, fusion of the epiphyses, growth of the genital organs and secondary sex characteristics are sex-hormone-dependent (Williams, 1974). Generally sex differences exist for both height and weight (Marshall, 1970). Medicinal treatment is a second major set of variables which can alter growth in the chronically ill child. Various drug therapies have been increasingly suspected of iatrogenic growth complication. For example, chronic (1.0 yr. +) methylphenidate treatment of hyperactive children has been demonstrated to suppress growth (Mattes and Gittelman, 1983). In addition, clinicians have long noted growth inhibition as a prominent side-effect of corticosteroid therapy, yet the mechanism by which this effect occurs remains unknown (e.g., Haynes and Murad, 1980). A third major set of
growth modulators is those environmental and physiological stressors which may act upon the chronically ill. Adverse familial environments, including those marked by abuse and neglect, are now believed to be the cause of decreased growth in a group of children originally diagnosed as suffering from idiopathic hypopituitarism (e.g., Powell, Bra- sel, and Blizzard, 1967). When such a child is removed from those circumstances, there generally occurs a period of rapid "catch-up" growth and maturation (e.g., Talbot and Sobel, 1947). Potent physiological stressors may also impact on growth and may include nephrosis and heart disease (Cheek, 1968) among several other disorders (e.g., Cheek, 1968). The role of environmental and physiological stressors in decreased growth, thus also appears potentially significant, but has remained largely unexplored.

In laboratory animal models, the influence of selected growth-altering variables have been analyzed, usually in isolation. Gluco­corticoids have been shown to abate growth in various rodent studies. For instance, daily prednisolone treatment has produced dose-related systemic toxicities as indexed by marked decrements in total body mass of weanling male rats (Sewell, Gallus, and Nanry, 1982). Other studies have reported glucocorticoid effects on growth of the skeletal system, and of long bones in particular (Becks, Simpson, and Li, 1944). Additionally, as in children, a variety of environmental stressors have been associated with growth abatement in non-human subjects. Animals raised in darkness, or subjected to excessive noise, have shown growth inhibition (e.g., Eayrs and Ireland, 1950; Sackler, 1959). The effect of housing condition on growth has been repeatedly explored
(e.g., DeFeundis, 1975; Sobel, Zuppinger, and Joss, 1979). Crowded gerbils have been noted, for example, to weigh less and have higher cortisol levels than those housed in pairs (Hull, Kastaniotis, L'Hommedieu, and Franz, 1976). Population size has also been demonstrated to markedly affect growth in mice (Christian, 1955). Further, gender effects on growth in animals have been noted in a wide range of species. Reutter (1976), for instance, attributes sex-differences in rat size to a slightly more than one week longer cell proliferative period in males. In sum, animal studies have shown that, as in the human clinical case, factors such as gender, environmental stress, and drug treatment may act by themselves to produce decreased growth. Certain combinations of such variables have been studied, but the interactive influence of other variables-in-combination has remained obscure.

As noted, adverse living environments and glucocorticoid treatment can act alone to suppress growth; whether these will interact to yield additive or supra-additive growth decrement is not known. That housing condition can markedly alter other toxic reactions to various drug challenges, has been found repeatedly (e.g., Gallus, Sewell, Nearchou, and Gault, 1982; Poling, Kesselring, Sewell, and Cleary, 1983). A growth-altering interaction between glucocorticoid treatment and sex is also plausible since effects of corticosteroids are modulated by the sex hormones in various assays. For example, androgens are known to antagonize certain metabolic effects of the glucocorticoids (e.g., Rubenstein and Wayne, 1980). Further, human females are reported more prone to develop corticosteroid-induced psychological dysfunction than are males (Ling, Perry, and Tsuang, 1981). More generally, sex has
been demonstrated as an important determinant of drug action employing numerous other dependent measures. An example of this was found with parachloroamphetamine toxicity in mice, wherein a significant sex by dose interaction was noted (Kesselring, Sewell, Gallus, Stiger, and Nearchou, 1983). McGovern et al. (1981) have reported differential toxicity of the anti-neoplastic acivicin, as a function of sex in mice. Other interactions of drug by sex have been reported for hexobarbital and pentobarbital anesthesia, and for strychine and picrotoxin toxicity (see Kato, 1975). Thus, while the effect of sex on several drugs' actions has been demonstrated, potential interaction between glucocorticoid treatment and sex on growth rates has not been examined.

A related interaction which may affect growth is that between environmental stressors and sex. Studies have been conducted on the modulatory influence of gender on behavioral and physiological reactions to noxious stimuli, including over-crowding. In one such experiment, group-housed female rats consumed more alcohol and had higher corticosterone levels than did grouped males (Hannon and Bolter, 1980). Whether these stressors interact with gender to change growth is unknown. It is thus plausible that glucocorticoid treatment, sex, and environmental stressors together may interact to suppress growth, in at least an additive manner. Therefore, with rodent subjects, the present study explored the influence on prednisolone dose, sex of subject, and crowding in the home cage, as these acted alone and in concert, to modify somatic growth.
Subjects

Seventy-two male and female, post-weaning, Sprague-Dawley rats were employed. Mean body weight on day one of study was 117.3 ± 8.6 gm. Rapidly growing rats were selected in an effort to maximize potential evidence of systemic toxicity. Subjects were bred and raised in our colony and then were randomly assigned to various drug-environment conditions. Subjects were housed either individually or in groups of six, with unlimited access to rat chow (Rat Chow 5012, Ralston-Purina Company, St. Louis, MO) and water in a constantly illuminated colony room maintained at 24-25° C.

Apparatus

Rats were housed in stainless steel cages (15 cm wide, 22 cm deep, and 21 cm high) (Unifab Corp., Kalamazoo, MI) located in the colony room. A food hopper and water bottle were attached to each cage. Individual body weights were determined with a top-loading scale (Pel-ouze, model 1000).

Procedure

The experiment employed a "group design", wherein various, randomly selected groups of rodents were assigned to combinations of
three different treatment or subject variables, these being sex of subject, housing condition and dose of drug. Rodents were housed individually or six per cage for five days to establish baseline weights before receiving any injections. Housing conditions remained the same from then on. For 16 consecutive days, animals were weighed daily and then injected with one of three prednisolone acetate doses (0.0, 2.0, or 4.0 mg/kg) (see Table 1).

Table 1
The drug dosage, sex, housing condition, and number of subjects for each group tested.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Sex</th>
<th>Housing Condition</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>---</td>
<td>Male</td>
<td>Group</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Saline</td>
<td>---</td>
<td>Female</td>
<td>Group</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Prednisolone</td>
<td>2.0</td>
<td>Male</td>
<td>Group</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Prednisolone</td>
<td>2.0</td>
<td>Female</td>
<td>Group</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Prednisolone</td>
<td>4.0</td>
<td>Male</td>
<td>Group</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Prednisolone</td>
<td>4.0</td>
<td>Female</td>
<td>Group</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Saline</td>
<td>---</td>
<td>Male</td>
<td>Individual</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Saline</td>
<td>---</td>
<td>Female</td>
<td>Individual</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Prednisolone</td>
<td>2.0</td>
<td>Male</td>
<td>Individual</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>Prednisolone</td>
<td>2.0</td>
<td>Female</td>
<td>Individual</td>
<td>7</td>
</tr>
<tr>
<td>11</td>
<td>Prednisolone</td>
<td>4.0</td>
<td>Male</td>
<td>Individual</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>Prednisolone</td>
<td>4.0</td>
<td>Female</td>
<td>Individual</td>
<td>7</td>
</tr>
</tbody>
</table>
Drug Preparation and Administration

Stock suspensions of prednisolone acetate (50 mg/ml) (Carter-Glogan, Glendale, AZ) were diluted with physiological saline to concentrations of 2.0 and 4.0 mg/ml and administered at 1.0 ml/kg volumes by intraperitoneal injection. These doses were chosen as previous research has shown them active in various assays with rodent subjects (Silverman and Maori, 1979). In addition, the lower dose (2.0) is within the clinical range used in the treatment of various human pathologies (Graef and Cone, 1978).

Statistical Analysis

Daily absolute body weight data and net changes in weight across study were analyzed by use of three-way analysis of variance (ANOVA) techniques (Hopkins and Glass, 1978). Results were reported graphically as group means ± one standard error.
CHAPTER III

RESULTS

Effects of Prednisolone, Housing Condition and Sex on Absolute Body Weight

Figure 1 shows the effects of prednisolone, sex, and housing condition on absolute body weights taken on the final day of study. Weight was lower for group-housed females, at all dosage levels, than for the individually housed females and for all males. Male weights decreased as dosage increased and in those subjects that were group housed. Three-way ANOVA indicated the existence of significant effects regarding dosage level ($F(2,60) = 41.425, p < 0.000$), sex ($F(1,60) = 295.923, p < 0.000$), and housing X sex ($F(1,60) = 4.623, p < 0.035$). However, no significant effects were found for housing in isolation or for interactions of (a) Dose X Housing, (b) Dose X Sex, or (c) Dose X Sex X Housing.

Effects of Prednisolone, Housing Condition and Sex on Grams-Change from Baseline Weight

The interaction of prednisolone, housing and sex had marked influence on weight gain during the 16 days of study. As shown in Figure 2, growth, as indexed by weight gain, was less as dose of prednisolone increased, for both male and female subjects. Males gained more weight than females in all three drug treatments and both housing conditions. Statistical analysis via three-way ANOVA indicated highly significant
Figure 1. Effects of sex of subject, housing condition (individual vs. grouped - 6/cage), and prednisolone treatment (0.0, 2.0, or 4.0 mg/kg) administered daily for 16 days, upon absolute body weights. Each data point is a group mean with standard errors too small for graphic representation.
Figure 2. Effects of housing condition (individual vs. grouped), sex, and drug dose (0.0, 2.0, or 4.0 mg/kg prednisolone) upon average changes in body mass, across the 16 days of study, for each treatment group. Each bar represents a group average, plus one standard error of the mean.
effects of dosage level \( (F (2, 60) = 39.097, p = 0.000) \), sex \( (F (1, 60) = 283.488, p = 0.000) \), as well as a significant dose X housing interaction \( (F (2, 60) = 4.833, p = 0.011) \) was evident. Further, a significant dose X housing X sex interaction \( (F (2, 60) = 4.833, p = 0.011) \) was evident. No significant influences were revealed, however, for (a) housing in isolation, (b) dose X sex interaction, or (c) housing X sex. Figure 3 shows the difference in mean weights for male and female subjects for both housing conditions, and the three drug levels. In all cases the males weighed more than the females. Body weight for males was greater in subjects that were individually housed than for those that were group housed in the lower dose group (2.0) and saline-treated subjects.
Figure 3. Effects of prednisolone treatment (0.0, 2.0, or 4.0 mg/kg) on mean absolute weight difference between sexes in grouped and individual housing conditions. For each comparison, male weight was greater than female.
CHAPTER IV

DISCUSSION

Daily prednisolone yielded systemic toxicities as evidenced by marked growth decrements, across the 16 days of study. Weight loss accrued as number of drug treatments, and as dose increased (see Figures 1, 2, and 3). This general result corroborates several previous reports of growth-detrimental effects in both children (Blodgett et al., 1956; Falliers et al., 1963; Liddle et al., 1974; Thomas and Mawhinney, 1973; Sobel, 1956) and laboratory animal subjects (Becks, Simpson, Li, and Evans, 1944; Parmer, Katonah, and Angrist, 1951; Sobel, 1958; and Wells and Kendall, 1940; Sewell et al., 1982). The mechanisms by which the glucocorticoids affect growth appear numerous and are disparate enough such that no unitary hypothesis of action is possible at this time (e.g., Melby, 1977). One well-known set of glucocorticoid effects is on protein metabolism (e.g., Liddle and Melman, 1974; Loeb, 1976; Baxter and Forsham, 1972). Glucocorticoids markedly enhance amino acid metabolism by (a) increasing mobilization from extra-hepatic tissues, and (b) incrementing synthesis of catabolic enzymes (e.g., Haynes and Murad, 1980; Parson, Crispell, and Ebbert, 1952). In reaction to high circulating glucocorticoid levels, protein synthesis is inhibited (Baxter et al., 1972; Clark, 1953; Deane, 1962), possibly in relation to observed decrements in extrahepatic RNA formation (e.g., Guyton, 1979) and/or amino acid uptake (Baxter et al., 1972; Clark, 1953; Ganong and Martini, 1973). Negative nitrogen balance
(Siber and Porter, 1953) may be due in part to amino acid deamination (Baxter et al., 1972; Parson et al., 1952). Altered protein metabolism is thus suggested as one important mediator of the prednisolone-induced weight losses here reported.

A second major effect of glucocorticoids on growth is osteoporosis that occurs as a consequence of catabolism and anti-anabolism of bone's protein matrix (Guyton, 1979). An imbalance in which bone destruction exceeds new bone formation is a result of glucocorticoid-induced reduction of RNA and protein synthesis in collagen-synthesizing cells (Talmage, Owen, and Parsons, 1975). Glucocorticoids further decrease bone growth via depression of calcium uptake by intestine and inhibiting calcium reabsorption by kidney (Salhanick, Kipnis, and Vande Wiehle, 1969). Another skeletal effect is the direct inhibition of mitosis in osteoprogenitor cells (Rassmusen and Bordier, 1974). In addition, glucocorticoid treatment may result in premature closure of the epiphyseal plates of long bones, thus yielding an irreversible shortening of stature (Liddle et al., 1974).

A third means by which glucocorticoid therapy may lead to growth retardation is the inhibition of growth hormone secretion. Large doses of glucocorticoids reduce somatotrophin (STH) secreted, amount of somatomedin formed in response to STH administration, and effect of somatomedians on cartilage (Pecile, Mueller, 1972; Daughday, Harrington and Phillips, 1975). Further, it is possible that amounts of glucocorticoid sufficient to promote such changes are released by the adrenal cortex in times of stress (Martin, 1976).

In the present experiment, one-half the subjects were group-housed
in an attempt to assess the influence of crowding stress. Housing was found to interact with sex and prednisolone dose in determination of body weight. Inspection of Figures 1 and 3 clearly display this result. A main effect of housing per se, was not found, however, and this later result is in keeping with previous work (e.g., Sobel et al., 1979; Latane, Cappell, and Jay, 1970). That some relation exists between environmental aversive stimuli and growth rates may have been inferred from assorted clinical reports of "psychosocial dwarfism", or the "failure-to-thrive" syndrome (e.g., Fried, 1949; Patton and Gardner, 1962; Talbut et al., 1947; Powell, Brasel, and Blizzard, 1967). Crowding stress has often been implicated in growth inhibition in animals (Jean-Faucher, Berger, Deturckheim, Veyssiere, and Jean, 1981; Hull et al., 1976). Other aversive environmental stimuli have also been noted to yield growth decrements including hypozia (Hunter and Clegg, 1973), excessive noise (Sackler, 1959), and darkness (Eayrs and Ireland, 1950).

Christian (1955) attributes a decline in body weight which accompanied increasing population density to a general non-specific reaction to crowding. Various studies which have employed dense housing have also shown increased adrenal and decreased gonadal activity. There are several plausible mechanisms by which crowding stress may interfere with growth. It is reasonable, for instance, that crowding may have been sufficient to produce changes in STH secretion and somatomedin formation and/or release, directly. This effect may have been further enhanced by aforementioned glucocorticoid influences.

A second plausible mechanism has to do with crowding having decreased food and/or water consumption in the group-housed, as compared
to individually housed subjects. That food and water remained continually available to all subjects decreases this likelihood, still, dominance hierarchies do develop in group-housed rodent populations and these may alter consumption patterns.

A third plausibility is that crowding stress may yield central nervous system effects which then change secondary metabolism via anterior pituitary mediation (e.g., of thyroid or gonadal effects). These plausibilities require examination.

Sex of subject was also an important factor in the growth changes here reported. Figures 1 and 2 demonstrate that within each sex, as dose of prednisolone increased so too did the amount of growth decrease. Thus, the present study extended to females the previous finding in weanling males that prednisolone inhibits growth (Sewell et al., 1982). Figure 2 shows also, however, that for each comparison, male weight was greater than that for females. Figure 3 emphasizes that this average weight difference was a clear function of both housing condition and dose, and that as dose increased, the degree of this sex difference abated. Statistical analysis revealed a significant 3-way interaction between sex, housing condition, and dose. Thus, the degree of sex difference was dependent upon environmental context, and this environmental influence was negated by increased prednisolone dose (see Figure 3). The mechanism(s) by which gender interacts with housing and prednisolone dose to determine growth rates remains speculative. However, it is plausible that differences in sex hormones are relevant. For instance, testosterone, an anabolic steroidal hormone, promotes (a) retention of nitrogen and electrolytes, (b) protein synthesis, and
(c) growth and strengthening of skeletal muscle (Martin, 1976). Animal crowding studies which show an increase in testicular weights, show a decrease in plasma testosterone levels (Jean-Faucher et al., 1981). High circulating glucocorticoids during development can alter testicular morphology and function (Paulsen, 1974). Lowered testosterone levels as a result of crowding stress and glucocorticoid treatment, may have been a gender-dependent cause of the growth retardation here reported. This possibility awaits analysis, perhaps through castration and androgen replacement procedures. Estrogen levels may also be affected by stressful events. A variety of stimuli, including rape, have been effective in producing spontaneous ovulation (Balin, 1972). During spontaneous ovulation, estrogens rise at a critical rate (Greep, 1973). In response to excess estrogen, cartilage growth is decreased and the epiphyses of the long bones calcify prematurely (Williams, 1974). Further, high doses of estrogen decrease growth by reducing somatomedian synthesis but do not inhibit its action (Pecile et al., 1972). Sex hormones thus can have a decided effect on growth.
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