

RATE OF LITTER SURVIVAL FROM STRESSED  
MOTHERS THROUGH THE F<sub>3</sub> GENERATION

by

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## INTRODUCTION AND LITERATURE REVIEW

The rise and fall of small mammal populations have been the center of much study and speculation, and the reasons for periodic fluctuations have raised much controversy. Many different factors have been implicated as sources of regulatory mechanisms of the populations, with environmental relationships being the most striking and probably the least understood. Population crashes and the resultant build up have been predicted with a certain amount of reliability after the study of combinations of various factors any of which may not be able to cause the crash themselves, but in combination may cause a devastating effect to the population. In many cases, it appears that no environmental condition known to man at the time could have caused the severe mortality and slow recovery observed.

Crew and Mirskaia (1931) obtained results that indicated the death rate, reproductive rate, and fecundity of a population to be adversely affected by population density; and suggested that such effects were due to individual response to the population density rather than an overall population response. Kott and Robinson (1963) found that increased density resulted in decreased litter size in late summer. Christian (1956) noted that reproductive rates of house mice declined as population density increased. Patric (1962) observed embryo counts in Clethrionomys to be inversely proportional to the population density with the smallest litters being produced during periods of high populations. Clarke (1955) observed large populations had shorter breeding seasons, decreased fertility, and lower infant survival rate and ascribed these effects to strife caused by over-crowded conditions. Davis (1951) determined that increasing and decreasing populations of Norway rats have greater pregnancy prevalences than do stationary populations. There was a greater mortality of young

at or shortly after birth in the increasing populations, but at the same time, greater survival of the young to produce the increase. Southwick (1955) found that birth rates in house mice gradually declined with continued crowding, possibly associated with reduction in fecundity of the mice accompanied by a reduction in food consumption despite an abundance of food. Other causes of reduced birth rates could be excessive population pressures due to the crowding, or failures in early embryonic development. Christian (1956) concluded that with increasing population density of Mus musculus there were increasing levels of stress reflected by adrenocortical hypertrophy and reproductive suppression in both sexes. Christian and LeMunyan (1958) found progeny nurtured by crowded mothers exhibited suppressed growth for at least two generations. They concluded that quantitatively or qualitatively deficient lactation resulting from crowding was the direct cause of growth repression. Cole (1956) stated that increased population density resulted in a decline or failure of the zygotes to implant properly, increased post-implantational intra-uterine mortality, post-parturient death or failure of the young to mature properly.

Although all preceding authors stated negative relationships between population density and survival, Howard (1961) found crashes in gopher populations to occur irrespective of the density of gophers present, but stress from intraspecific competition might in some way have been a contributing factor. Godfrey (1955) could find no environmental factor adequate to explain the extreme population reductions observed in Microtus agrestis. He thought the crucial factor to be some carry over from the preceding year.

Age structure of the population has a definite effect on the reproductive rate of meadow mouse populations, since the size of the litters

varies with the size (age) of the females (Beer and MacLeod, 1961). Kott and Robinson (1963) found this to be also true in Microtus agrestis and Mus musculus. Young breeding mice of both species apparently have smaller litters than older mice. This is especially reflected after emerging from winter hardships. In many cases, though, the young produce large litters in the spring enabling the population to expand rapidly; and as the population fills the habitat, litter sizes decrease. Beer, MacLeod and Frenzel (1957) found the potential litter size to be smaller in small (young) females than in larger (older) females. In this case it was reflected in a considerably higher rate of total intra-uterine loss in the small females. Lack (1954) also showed age to be of some importance.

Parkes (1926) observed the size of the litters affecting inversely the individual size of the young. The quantity of milk available to each young was increased by reducing the litter at birth (MacDowell, Gates and MacDowell, 1930), and thus the growth rate was accelerated.

Availability of water plays an important part in the survival of the young as Lindeborg (1950) observed with Peromyscus maniculatus bairdi. Water consumption was found to increase as parturition nears. One day before parturition, 36% more water was consumed by pregnant mice than by non-pregnant controls. Water consumption increases further after parturition to 111% at 15 days post-partum and 158% at 22 days. If water deficiency occurs at a critical time, the young may not survive. Usually the litter size is decreased to compensate for the water deprivation, even to complete destruction of the litter.

The kind and amounts of food available at different times during pregnancy and lactation either inhibits or stimulates the growth of a population. Results of reduced food could be traced to prenatal maternal

effects, postnatal maternal effects, inherent growth potential, and non-maternal environmental effects (Bateman, 1954). These effects contributed approximately equally in the production of variation in weights of single young and were accentuated by increasing the size of the litters.

Lack (1954) observed that litter size in Microtus agrestis can be affected by available food as well as many other factors mentioned before. Seasonal changes in available food appeared to be the most important limiting factor (Brown, 1953). Lauckhart (1957) thought the effects resulted from nutritional deficiency rather than an actual starvation. Such malnutrition would be reflected in increased susceptibility to disease, lowered reproduction and reduced survival of young; all of which are symptoms of cyclic declines. Strecker and Emlen (1953) reported results with Mus musculus generally confirming Lack's conclusions. During a food crisis there was no unusual loss of adult animals or of young born before the crisis. The animals surviving showed no loss in weight or viability, but feeding rhythms were altered. It was suggested the physiological response be interpreted in terms of the shifting of adaptation energy under stress from reproductive to survival functions.

Experimentation with different food stuffs has given a highly diversified view of what can happen to the individual and in turn to the population. French, Ingram, Knoebel, and Swift (1952) observed a significant decrease in reproductive performance of rats receiving a 23% fat diet when compared to 4.4% fat. This was manifested in the birth of smaller numbers of lighter weight young in the low fat group. The addition of carbohydrate in combination with the fats resulted in a significant improvement in lactation. A decrease in reproduction also occurs when the pregnant female is fed different food stuffs. When one food is suddenly substituted for

another, it is difficult to insure that the mice eat sufficient amounts of the new food. "Such treatment is unlikely to cause deficiency in the accepted sense of the term, but is more likely to cause some type of physiological stress reaction" (McClure, 1959). Maynard and Rasmussen (1942) found that by increasing the fat in the normal diet of rats as high as 9% resulted in larger gains of sucklings than a similar diet containing 4.5% fat. Purified diets containing 18% fat gave superior results.

Slonaker and Card (1923) showed that diets composed entirely of vegetable material resulted in a greater number of rats becoming impotent, reduced fecundity, and loss of power of reproduction by the third generation. Animals still capable of reproduction were restored to nearly normal conditions within two generations by an omnivorous diet containing animal protein. The effects of the shock of a restricted diet were sometimes not seen for several months. Mueller and Cox (1946) analyzed the milk of rats fed diets of different compositions and found increases in the protein intake resulted in increased milk yields, but showed no significant change in the percent protein in the milk. High calcium diets resulted in decreases both in milk volume and in the percents of calcium and phosphorus in the milk, with consequent decreases in the total amounts of calcium and phosphorus secreted. High phosphorus diets had no demonstrable effect on these milk constituents. Mueller and Cox also reported the average number of young raised on low protein diets was only slightly less than the number raised on high protein diets, although their weight was markedly reduced. Junqueira and Schweigert (1949) reported meat protein added to the diet to be the major factor capable of improving growth of young. Curtiss (1953) found low protein intake resulted in hypoproteinemia, mammary underdevelopment, and small fetuses. Bernhard (1961) showed that

generally the milk supplied by the females contained an amount of protein adequate for maximal growth but not much more. Nelson (1959) observed that dietary protein must be above certain minimal levels for maximal growth of the young in litters of particular sizes.

Another factor that may decrease the volume or quality of milk might be the B vitamins (Mueller and Cox, 1946). Decreasing the intake of yeast decreased the volume of milk, the size of nursing young, and weights of lactating mothers with significant changes in the composition of the milk. It is not known at the present time whether the effects result from decrease in the volume or the quality of the milk. In this particular experimental procedure, it was thought to be the quantity.

Extremes, either heat or cold, may play an important part in reduction of fecundity. Macfarlane, Pennycuick, and Thrift (1957) reported a hot environment brought about high fetal loss. Resorptions rose to 75% at 25° C as compared with 58% at 22 to 23° C. These losses were decreased by giving supplements of vitamins and protein.

Eskridge (1955) and Howard (1951) determined that small rodents can withstand prolonged exposure to freezing temperatures only if adequate food is available. Peromyscus maniculatus survived in a refrigerator maintained at 0° C for as long as six weeks when confined in individual cages with adequate food and no nesting material. If food was withdrawn, they would die within a few hours. This suggests these rodents cannot convert protein or fat into oxidizable substrate fast enough to meet the increased metabolic requirements imposed by cold and starvation. Survival could be increased by placing several animals together facilitating huddling (Sealander, 1952). Howard (1951) and Eskridge (1956) showed rodents unable to survive overnight when exposed to freezing temperatures without food or

nesting material. When nesting material was provided, small rodents required less food to maintain their body temperature and survived one or more days at freezing temperatures without food. Resistance was increased by acclimitizing the animals to the cold beforehand (Prychodko, 1964). Isolated mice provided with nesting material survived somewhat lower temperatures than isolated animals without shelter. The survival of both groups at 4° C was higher than in the controls which were kept in groups before being exposed to low temperatures. It was concluded that during isolation at room temperature the animals developed greater capacity for heat production enabling them to compensate for the increased heat loss at low temperatures.

Calhoun (1949) indicated social conditioning of Norway rats may be a potent factor in population control. The socially dominant, larger females weaned 10 out of 12 litters, while the socially inhibited rats weaned only one out of 12 litters. The histories of the mothers indicated a physiological and psychological disturbance in socially inhibited individuals which may have had a deleterious effect on the progeny either through poor fetal nutrition or from a breakdown of maternal instincts.

Nearly all the work that has been done with fluctuations of small mammal populations has been recorded only for the immediate generation under stress. Responses of the population to the effects of some factor causing this fluctuation has seldom been carried on to succeeding generations. Chitty (1952) theorized that strife during the breeding season resulted in early death of the young, and those surviving were abnormal from birth and were more susceptible to various mortality factors; and these defects in a more severe form were transmitted to the next generation. Christian and Lemunyan (1956) observed intra-uterine or post-parturient

factors adversely affecting the ability of surviving progeny to reproduce, seriously affected the future of their populations. Inadequate nutrition during nursing, or intra-uterine physiological derangements, conceivably could produce such manifestations. Christian (1956) suggested there was a suppression of lactation in house mice because of dense populations. Obvious suppression of progeny nurtured by the crowded mothers persisted for at least two generations and was interpreted by Christian to be due to quantitatively and/or qualitatively deficient lactation resulting from the crowding.

Most attention has been given to causes of population "crashes" or less violent declines. Slow recovery of a population after a crash has been noted in most reports involving entire cycles, but little effort has been expended to explain the inability of a population to recover quickly from the effects of the crash. Chitty (1952) suggested a prolonged debilitation of the young surviving the crash, and Christian and LeMunyan (1958) showed that stress from overcrowding had a depressing effect on reproduction for at least two generations.

In an effort to observe first hand the effects of specific stress factors to a population and to test the theory of Christian and LeMunyan (1956), that the effects lasted for several generations, the following set of experiments were conducted.

#### MATERIALS AND METHODS

White mice of the Swiss strain were used. The animals were housed in stainless steel cages with exterior dimensions of 20 X 15 X 27.5 cm. For breeding purposes, two females and one male were placed in each cage. When the females appeared pregnant, they were removed from the cage and

placed in a separate cage by themselves with a fine wire mesh bottom with wood shavings for nesting material. The diet of the animals consisted of a proven laboratory mix containing 22% protein, 7% fat and an excess of all known required minerals and vitamins. All feeds were compounded by the Kansas State University Milling Department.

Originally, the animals were divided into five groups; one control group and four different experimental groups. The first group, designated "Water experimental", was treated in the manner described above, but at the time of observing the copulation plug they were placed on a 2/3 water requirement. This method proved unsatisfactory and was abandoned. In an effort to find some method with water as a stress factor, the animals were placed on a 2/3 water requirement at the time of parturition; and again this proved fruitless because of the high variability in the requirements of different females of the amounts of water consumed at different times after parturition.

In the second experimental group, designated "Cold from copulation plug", the animals were treated the same as the controls with the exception they were placed in a refrigerator at 2-3<sup>0</sup> C for one hour a day from the time of observation of the copulation plug. This method had some desirable results, but again was not completely successful because the presence of a copulation plug does not necessarily mean fertilization has occurred, and the treatment further reduced pregnancies below the level necessary to conduct an accurate experiment.

The third experimental group was designated "Cold after parturition". In order to improve on the cold stress method, the animals were not placed in the cold environment until after parturition. The conditions were the same as the preceding group; one hour a day at 2-3<sup>0</sup> C. This was continued daily until the twentieth day after parturition. Much preliminary work was

needed to find a temperature that allowed some survival of young but not complete survival. The females, while in the cold, were isolated in wire baskets without nesting material.

The fourth experimental group, designated "Low protein", was placed on a protein deficient diet during the day of parturition. The diet consisted of equal parts of yellow corn meal and corn starch with a concentrate of minerals and vitamins adequate to supply all known needs other than proteins.

To compare the experimental results, the number of young observed during the day of parturition was recorded. No attempt was made to weigh the young until the fifth day because moving the young had some adverse effect on the animals and the mother killed them in a high percentage of the cases. Weights were taken on days five, 10, 15, and 20. Records were maintained on individual mice so age at parturition, time between parturitions, weights at different times, and survival of young were obtainable on all experimental animals. The differences were analyzed by analysis of variance statistical procedure.

## RESULTS

Controls (Group I). From the beginning of these experiments, problems of achieving reasonable conception were encountered unless one male was housed with one female. In order to increase the copulatory rate in cages with two or more females, males were rotated as soon as a copulation plug was observed. The male usually copulated with one female and would not copulate with the other female unless the females were separated. Even though copulation was not observed, one female appeared "dominant" and did not allow copulation with other females. Rotation of the males nearly always insured copulation with the next female to come into estrus. If

the female was removed immediately after copulation, the male would then copulate with the other female. If a new female was introduced into a cage with the remaining female and male, the male would almost always copulate with the remaining female as soon as she came into estrus rather than with the new female. The best results were obtained by changing females and males to other cages so "dominance" could not become established.

The control group contained 33 females that gave birth to the F<sub>1</sub> generation containing 213 young of which 66 percent survived. This low rate of survival was due primarily to excessive failures of litters during the period February 4 to March 10. Animals born during this period had a high mortality rate, although the animals surviving were nearly normal in weight. Litters born before and after this period survived well.

The individual pup weights averaged 2.7 gm at five days, 4.7 gm at 10 days, 6.4 gm at 15 days, and 8.8 gm at 20 days (Fig. 1). Animals surviving the first ten-day period usually survived to the last day of weighing. The highest losses occurred immediately after parturition because the female failed to care for the young or killed them by biting them on the back of the neck. Removal of dead animals from the cage proved of value in reducing further killing in nearly all the litters in which some young were killed. The normal rate of growth for all controls averaged nearly 2 gm for each five-day period, with the largest gains between the fifteenth and twentieth days. The heavier pups maintained a higher rate of growth than animals weighing less than average. Even litters with a larger number of young had the greatest growth rate near the end of the weighing periods. Litters with only two or three young at the fifth day had the greatest increase in weights near day ten, but then dropped off to maintain average rates.

Since small litters generally have individuals of larger size, total

average litter weights were computed (Fig. 4). The average litter weights were 19 gm at five days, 30 gm at 10 days, 38.3 gm at 15 days, and 50.8 gm at 20 days. Seventy percent of the females of the original control group produced litters by the time they were five months old.

Of the  $F_1$  control females, 23 gave birth to 171 young of the  $F_2$  generation of which 80 percent survived. The individual pup weights were 3.7 gm at five days, 5.5 gm at 10 days, 6.8 gm at 15 days, and 8.6 gm at 20 days (Fig. 2). The young in this generation were a little larger at birth but showed a slightly lower average weight gain than did the  $F_1$  litters. The greatest gains were made before the tenth day, then tapered off and showed another increase in rate between the fifteenth and twentieth days. The average litter weights were 28.4 gm at five days, 41.5 gm at 10 days, 50.3 gm at 15 days, and 62.9 gm at 20 days (Fig. 5), considerably higher than the  $F_1$  litters. Of the 23 litters born, only one litter failed to reach the end of the 20 day period, and in this case the female killed the young on the day of parturition. The surviving average litter weights showed a straight line growth with no significant changes in the rate of growth (Fig. 5). Nearly all animals surviving at day five were still alive at day 20. The greatest loss was on the day of parturition; most of the losses occurred before day 10.

Seventeen females of the  $F_2$  generation produced 188 young of the  $F_3$  of which 76 percent survived. The average individual pup weights were 2.9 gm, 4.3 gm, 5.7 gm and 7.3 gm at day five, 10, 15 and 20 respectively (Fig. 3). The rate of gain was nearly 50 percent in the 5-10 day period, dropping to 28 percent during the last weigh period. Seventy percent of the fatalities occurred before day 15. The average total litter weights were 30.6 gm, 40.6 gm, 50.9 gm, and 61 gm respectively (Fig. 6). All

## EXPLANATION OF FIGURES

Fig. 1. Average individual weights of  $F_1$  generation consisting of 91 litters with 593 young at birth and 325<sup>1</sup> young at end of weighing period. Heavy lines represent total weights divided by the number of young present at each weighing period. Thin lines represent total weights at each weighing period divided by the number of young on day of parturition.

Fig. 2. Average individual weights of  $F_2$  generation consisting of 95 litters with 815 young at birth and 543<sup>2</sup> young at end of weighing period. Heavy lines represent total weights divided by the number of young present at each weighing period. Thin lines represent total weights at each weighing period divided by the number of young on day of parturition.

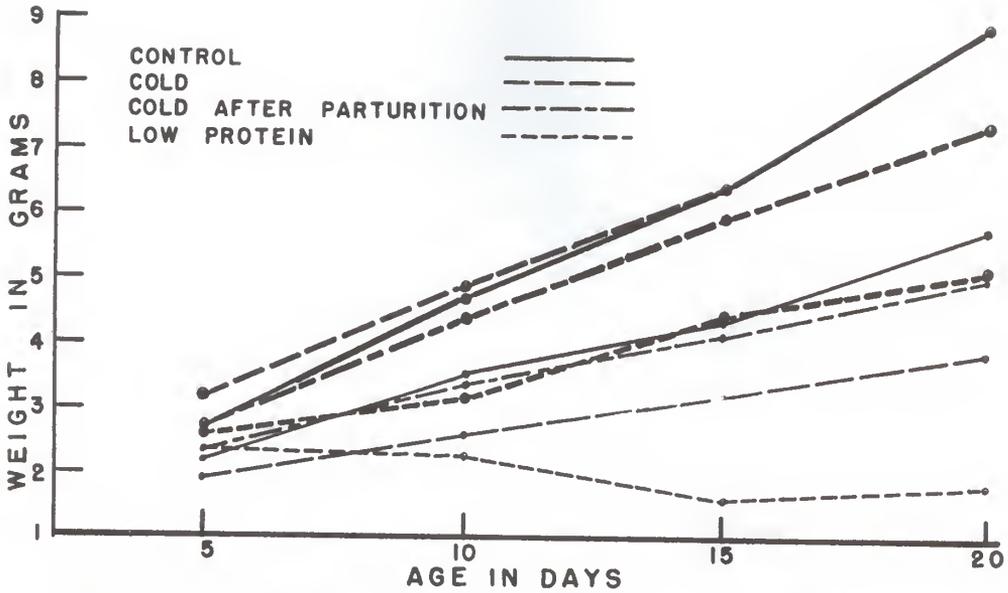


FIG. 1. F-1 GENERATION AVERAGE INDIVIDUAL WEIGHTS.

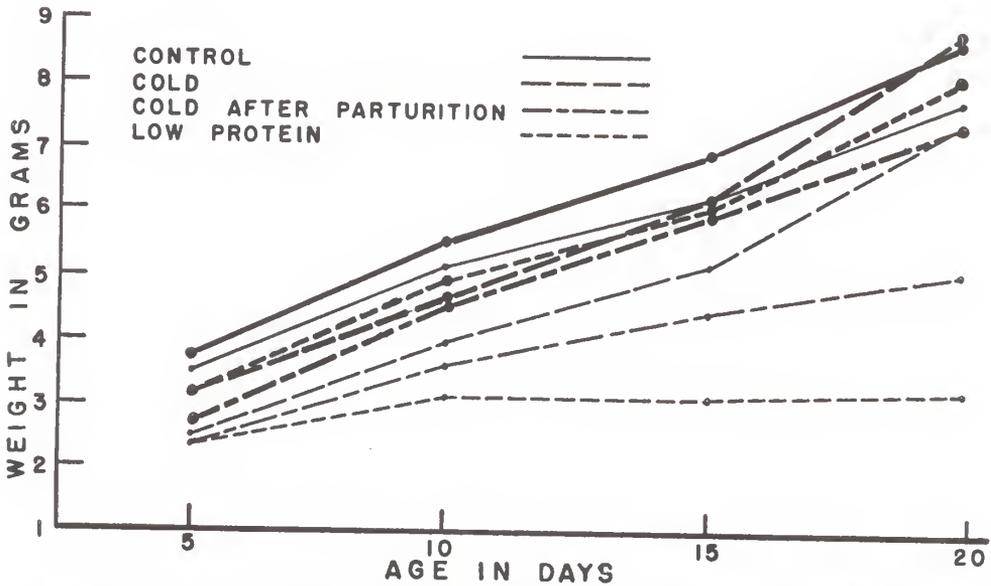


FIG. 2. F-2 GENERATION AVERAGE INDIVIDUAL WEIGHTS.

litters survived for the 20 day weighing. The average age of the females at parturition was 93 days.

Cold Group (Group II). Of 50 animals in which copulation plugs were observed during one period, only 22 animals gave birth to young. Six more animals were added later to bring the total to 28 which gave birth to 183 young of which 77 survived until day 20. The average individual weights were 3.2, 4.7, 6.4, and 8.8 gm at day five, 10, 15 and 20 respectively. Of 28 original litters only 14 survived for 20 days. The greatest loss of individuals occurred during the first five days after parturition. Animals surviving past this time showed a relatively high survival rate. The total litter weights were 20.5, 27.8, 37.0, and 49.4 gm at day five, 10, 15, and 20 respectively (Fig. 4). The average total litter weights showed a steady increase with no sporadic increases or decreases.

The average of the  $F_1$  generation at parturition was 126 days, 33 days longer than the controls. Eleven females gave birth to 70 young of the  $F_2$  generation of which 82 percent survived. The average individual weight of the survivors on day 20 was 8.7 gm, essentially the same as the controls. These animals showed a steady increase in weight until the fifteenth day; at this time a sharp increase in weights were observed (Fig. 2). All mortalities occurred before the fifth day. The average litter weights were 17.8 gm at day five, 26.9 gm at day 10, 35.7 gm at day 15, and 50.8 at day 20 (Fig. 5). Only one litter of 11 failed to survive until the last day of weighing.

Cold After Parturition (Group III). Nineteen females gave birth to 132 young. Of this number, 64 percent survived with the mother on daily cold stress. The average individual weights were 2.7, 4.4, 5.8, and 7.8 gm respectively (Fig. 1). Most of the mortalities (32 of 40) occurred before

#### EXPLANATION OF FIGURES

Fig. 3. Average individual weights of  $F_3$  generation consisting of 51 litters with 540 young at birth and 331 young at end of weighing period. Heavy lines represent total weights divided by the number of young present at each weighing period. Thin lines represent total weights at each weighing period divided by the number of young on day of parturition.

Fig. 4. Average litter weights of  $F_1$  generation consisting of 91 litters being produced and 57 litters present at end of weighing period. Heavy lines represent total weights divided by the number of litters present at each weighing period. Thin lines represent total weights at each weighing period divided by the number of litters on day of parturition.

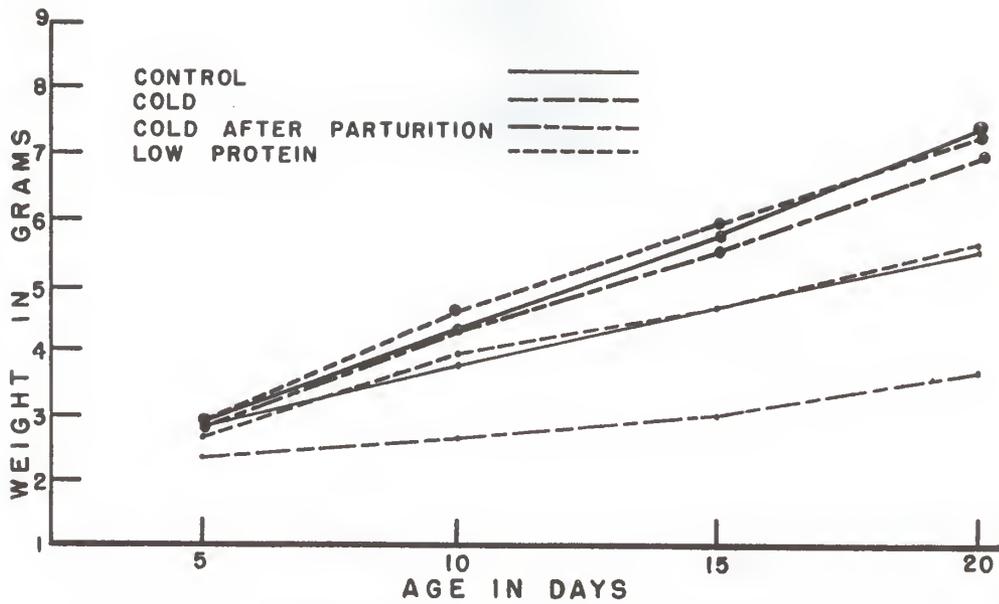


FIG. 3. F-3 GENERATION AVERAGE INDIVIDUAL WEIGHTS.

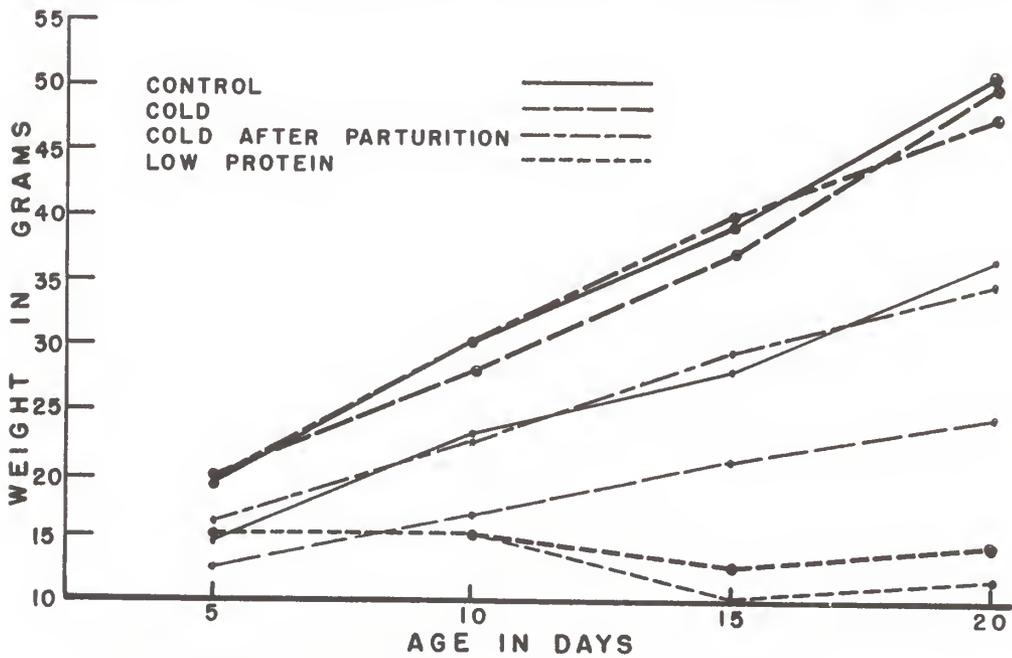


FIG. 4. F-1 GENERATION AVERAGE LITTER WEIGHTS.

## EXPLANATION OF FIGURES

Fig. 5. Average litter weights of  $F_2$  generation consisting of 95 litters being produced with 78 litters present at end of weighing period. Heavy lines represent total weights divided by the number of litters present at each weighing period. Thin lines represent total weights at each weighing period divided by the number of litters on day of parturition.

Fig. 6. Average litter weights of  $F_3$  generation consisting of 51 litters being produced and 43 litters present at end of weighing period. Heavy lines represent total weights divided by the number of litters present at each weighing period. Thin lines represent total weights at each weighing period divided by the number of litters on day of parturition.

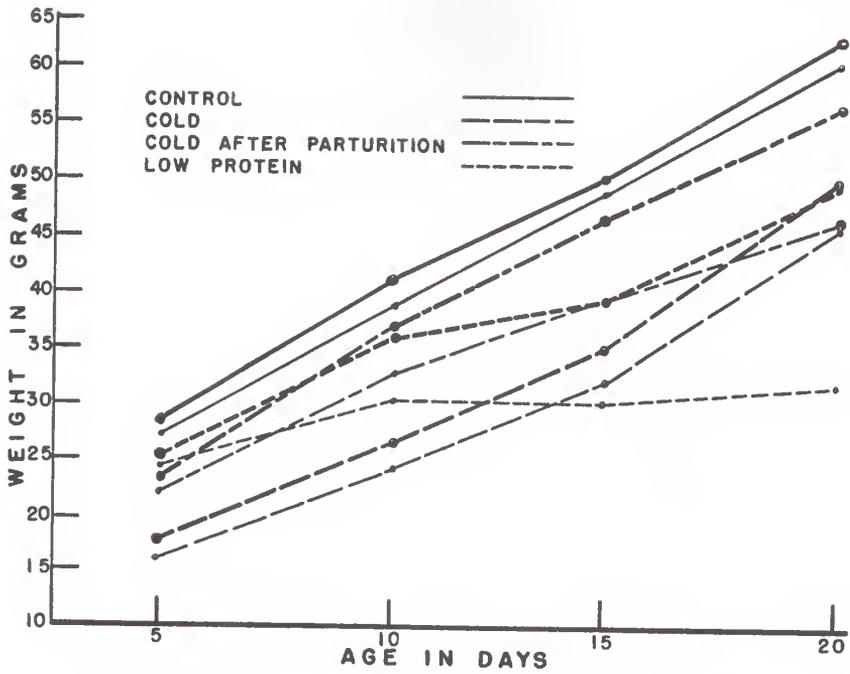


FIG. 5. F-2 GENERATION AVERAGE LITTER WEIGHTS.

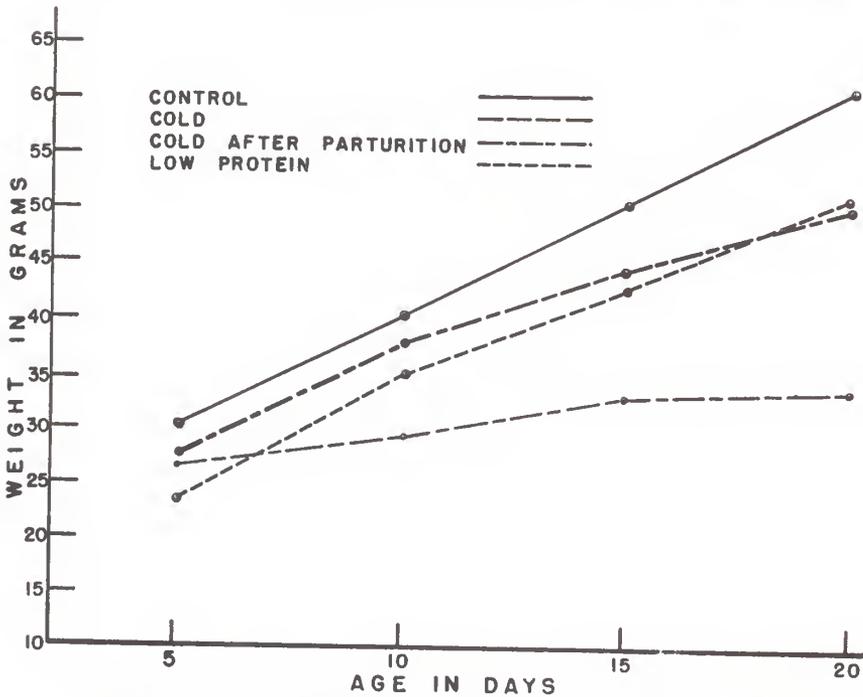


FIG. 6 F-3 GENERATION AVERAGE LITTER WEIGHTS

the tenth day. The average litter weights were 19.9, 30.5, 38.4, and 47.2 gm respectively (Fig. 4). The total litter weights increased at a very steady rate. Females of the  $F_1$  generation averaged 115 days of age at parturition. Forty-four females produced 407 young of which 69 percent survived. Only 15 percent of the females capable of producing young in the five month period did so (Fig. 7). The individual weight at day 20 was 7.3 gm, 17 percent below controls. Loss of young occurred throughout the weighing period with the highest mortality before the fifth day. Total litter weight on day 20 was 56.6 gm or approximately average for all control groups (Fig. 5).

The  $F_2$  generation averaged 109 days of age when they produced young. Twenty-three females gave birth to 251  $F_3$  generation young of which 44.2 percent survived. The average individual weight was 6.9 gm or 24 percent below control averages on day 20 (Fig. 8). Animals were killed throughout the weighing period, mostly between the fifth and tenth days. The average litter weight on day 20 was 50.9 gm, a little below average for controls (Fig. 6). Fifteen of the 23 litters survived to weaning. Total litters were maintained intact after the fifteenth day, and most litters not surviving were lost before the tenth day.

Low Protein (Group IV). Eleven litters containing 65 young were put on the low protein diet, but only 35 percent of these survived. The average individual weights were 2.5 gm, 3.2 gm, 4.4 gm, and 5.1 gm on days five, 10, 15, and 20 respectively (Fig. 1). Significant drops in rate of increase occurred between the tenth and fifteenth day. Individual weights showed a sporadic decrease and increase through the period. Average litter weights decreased progressively from 15.5, 15.2, 12.7, to 14.5 gm because of drastic reduction in numbers of pups coupled with low individual growth increments. The pups survived relatively well until the tenth day after which mortality

became excessive with 65 percent perishing.

By the end of the fifth month following birth, only 10 percent of the  $F_1$  females had produced young of the  $F_2$  generation (Fig. 7). Three females bred in the expected time interval, but all young perished within one day after birth. Over six months elapsed before these animals bred with any regularity.

Seventeen of the  $F_1$  females produced 167 young of which 40.7 percent survived. The individual average weights of the  $F_2$  survivors were 3.2, 4.8, 6.0, and 8.0 gm respectively (Fig. 2). Animals succumbed throughout the weighing period with highest mortality rate before the fifth day. The

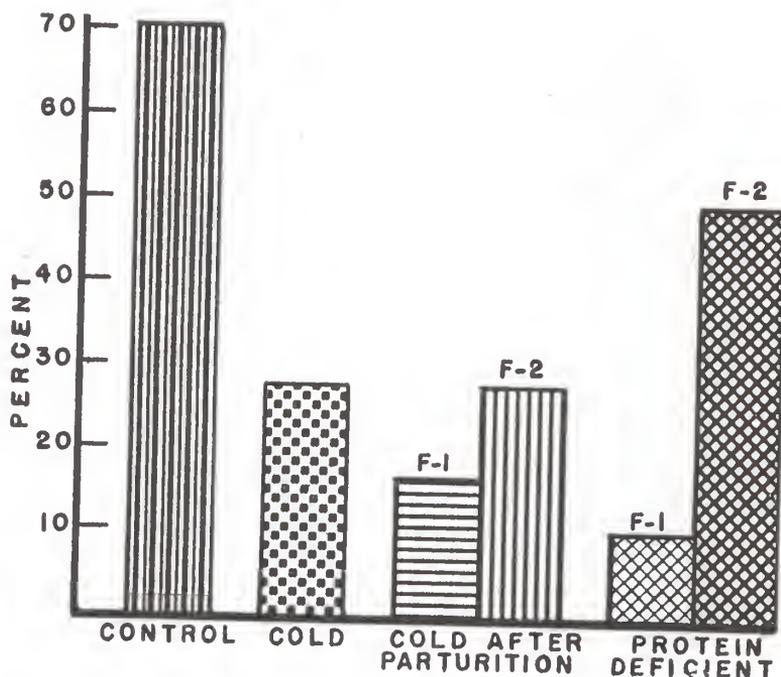


Fig. 7. Percent of animals producing young before five months of age, determined by number of females placed with males divided by the number of females that produced litters. Animals of  $F_1$  and  $F_2$  within Groups I and II were considered together because there was no difference between generations.

average surviving litter weight at day 20 was 49.6 gm (Fig. 5), or only slightly below the controls. At 120 days these animals were 2 gm smaller than the controls.

The  $F_2$  generation averaged 114 days at parturition. No unusual problems in breeding these animals were encountered. Eleven females produced 101 young of which 79 survived. The individual weights were 2.9, 4.6, 5.9, and 7.2 gm respectively (Fig. 3). The total litter weights were 23.9, 35.8, 42.7, and 51.7 gm respectively (Fig. 6). The total litter weights showed a slight increase at day five and then a steady gain in weight until the last day of weighing. There was no loss of total litters. The 120 day weights were the same as the controls. Forty-nine percent of the females produced young before they were five months old (Fig. 7).

#### DISCUSSION

The average individual weights of all groups of the  $F_1$  generation were significantly different ( $P < 0.05$ ). The cold stressed group had the same average individual weights as the controls, 818 gm at day 20; but the survival rate of group I was 66 percent compared to 42 percent for group II. In order to take into consideration the survival rates as well as pup weights, the total number of young at the day of parturition was divided into the total litter weights at day 20, resulting in a somewhat lower average weaning weight in the  $F_1$  and  $F_2$  of all the experimental groups (thin lines in Fig. 1, 2).

The total litter weights of surviving young averaged 50.8 gm at day 20 for group I as compared to 49.4 for group II. Again, to consider survival as well as weight gains, the total number of litters born was divided into the total litter weights at day 20 (Fig. 4) giving 36.9 gm for group I

and 24.6 gm for group II (thin lines, Fig. 4). This method of computations illustrates a difference between the groups in contrast to the uniformity of those litters that survived. There was a distinct indication that a general compensating mechanism was involved by which litters were reduced to numbers that permitted reasonable maintenance, but some females were unable to accommodate to the stress and lost most or all of their young.

The low parturition rate (42 percent) of those females introduced into the cold-stress group after a copulation plug was observed distinctly shows that cold stress can be a factor in fetal loss. A high percentage of those that did not parturate were pregnant and the embryos resorbed; and in several cases, cystic uterine glands were observed. It appears that resorption of embryos constituted a response of the animal to the extreme cold conditions of the experimental procedure, and the cystic glands probably resulted from prolonged pseudopregnancy after embryo resorption. Many of the animals examined proved to have been 10 to 15 days pregnant at the time the embryos began to be resorbed. Approximately 50 percent of the animals examined showed evidence of resorbed embryos.

The animals in groups I (Control) and III (Cold after Parturition) showed no significant differences in the percentage of survivors, 66 percent compared to 64.4 percent in group III. The 20 day average individual weights were found to be significantly different ( $P < 0.05$ ). Dividing the total number of young parturated into the total of the litter weights gave 5.7 gm for the animals in group I and 5.0 gm for group III. The average weights of surviving litters were significantly lower in group III.

The group most affected was the low protein group (Group IV) with 35 percent survival compared to 66 percent for group I. The individual weights at day 20 were 8.8 gm for the controls and 5.1 gm for the animals

in group IV. The total number born divided into the 20-day litter weights are 5.7 gm for group I and 1.8 gm for group IV. The surviving animals of group IV were definitely stunted and did not recover for almost five months. Weights taken on the day 120 showed an average of 26 gm in group I as compared to 17 gm in group IV. Members of groups II and III showed no such lag in growth rates. A much longer recovery time was needed after such severe stress as the low protein diet. While all other experimental groups showed a slightly lowered growth rate at day five, the effect of the low protein diet was not noticeable before the fifth day of lactation. By the tenth day, the difference was quite significant; 4.7 gm for group I compared to 3.2 gm for the experimentals. The total litter weights show this same difference. The total average litter weights at day 20 of group I was 50.8 gm compared to 14.5 gm for group IV. Protein-stressed females maintained eight of ten litters, but lost a high percentage of young (Fig. 4).

In the  $F_1$  generation the effects of cold, whether administered before or after parturition had a significant effect on the offspring. This is especially manifested in the survival of young rather than individual weights of survivors. There seemed to be an attempt by the female to maintain a litter of normal individuals by reducing the number of animals in the litter. The protein deficient diet had the most devastating effect in the average litter weights. Not only was the percentage of young that survived very low, but the individual weights were also seriously affected. There was a fairly high percentage of litters surviving, but the animals that survived were seriously affected as shown by the time needed to grow to maturity; for the controls 92 days compared to more than 140 days for the protein experimentals.

In the  $F_2$  generation the average individual weights of all experimental groups except group II were significantly different from the controls

( $P < 0.05$ ). The survival rates of group II were also not significantly different from those of group I. The only significant difference found in group II was in the average total litter weights: group I averaged 62.9 gm compared to 50.8 gm for group II.

In this study, the best breeding time was in the winter and spring, with much lowered reproduction in the summer and fall. It was noted that females of group II ceased breeding almost two months earlier in the summer than did members of group I. The effects of the cold applied to the parental female had not completely diminished at this time and still had some effect on the animals.

The 20 day weights of  $F_2$  group III were significantly different from group I. Group I young averaged 8.6 gm compared to 7.3 gm for group III. The rate of survival was significantly higher in the controls, 80 percent, than in group III, 68 percent. The average total litter weight for group I was 62.9 gm compared to 56.6 gm for group III. In group I, 21 of 22 litters survived compared to 36 of 44 litters in the group III experimental group.

The animals in group IV again showed the greatest effect. The average individual weights on day 20 were significantly different for this group as was the survival rate, 80 percent for group I and 41 percent for group IV. The total average litter weights were also significantly lower than the controls, 49.6 gm compared to 62.9 gm. The average age at parturition was significantly greater in group IV,  $F_2$ . The effects of the low protein affected all stages of growth and maturation in this group.

The effects of the stress factors were still detectable in some groups of the  $F_2$  generation. The effects on the cold group (II) were almost completely gone with the only significant difference occurring in the total

average litter weights. Significant differences were found between the controls and the cold after parturition group (III), in the percentage of animals surviving, individual weights and average total litter weights. The individual weights of the low protein group were only slightly significantly different from the controls; the most significant differences occurred in the percentage of animals surviving and the average total litter weights. These observations lead to the assumption that the effects of less severe stress begins to diminish earlier than more severe factors such as the protein deficient diet.

The individual weights of the  $F_3$  generation of all groups were proven to not be significantly different from the controls. The average total litter weights were significantly different between the controls and groups III and IV. The most significant differences between group I and group III was in the percentage of animals surviving, 76 percent for the controls and 44 percent for group III. No significant variation was shown in the average individual weights, percentage surviving, average total litter weights or age at parturition in the members of group IV, indicating a complete recovery from the effects of protein deficiency stress by the third generation.

Much of the work completed in this study seems to support the work done by Christian and LeMunyan (1958). Stressed mothers produced young 15 percent lighter than animals not placed on stress. Small litters of one to five individuals were not significantly different from controls. Litters with five or more animals were more seriously affected, although some females with more than five offspring showed no apparent detrimental effects. Christian and LeMunyan used males from stressed litters and concluded from this that males were not affected because when placed in a breeding situation they were capable of reproduction. In this study, no stressed males

were used in any instance for breeding purposes.

Results obtained seem to confirm Christian and LeMunyan's observations that if females were stressed after breeding, losses occurred during the first half of gestation. Resorbed embryos were observed in the cold group that appeared to have developed to no more than ten days.

After parturition, the reasons for high mortality appeared in some instances to be due to failure of the female to care for the young. The young would be pushed out of the nest and allowed to die. In some cases, the female would care for only part of the litter, apparently in an attempt to produce at least part of a normal litter.

Christian and LeMunyan (1958) stated dominant females were better producers than females that showed subordinate activities. The results of this experiment support this observation. Unless dominant females were removed from the breeding cage, the males would not attempt to copulate with the subordinate female. Even when the dominant female would not copulate with the male, she would not allow the subordinate to copulate either; although all were allowed to huddle together during periods of rest or of cooler temperatures. Also noted by Christian and LeMunyan (1958), females capable of producing large litters were also capable of nursing them. In this study control as well as certain stressed animals producing litters of seven to ten individuals seemed to be able to produce milk in sufficient quantities to allow for maximum growth of the young. In nearly all cases, quantity of milk seems to be the restricting factor in pup growth. Animals producing large litters reduced the number of young in the litter to as much as half to allow the young surviving to grow at a normal rate. In these cases, the weaning weights were the same for the controls and the stressed groups. This reaction seems to be an individual response rather than a complete

population response.

Chitty (1952) stated that later progeny of stressed adults were abnormal from birth and were thus more susceptible to various mortality factors. This appears to be a correct assumption. The offspring in this experiment were smaller and less resistant to certain factors such as infestation by lice. The young of stressed mothers that became infested lost hair, developed skin sores and in some cases quit eating, while the controls showed few effects.

Young born from stressed animals were also harder to handle than controls. While this observer could pick up and handle controls with no fear of being bitten, the stressed animals were completely unpredictable in their behavior. They were much harder to catch and would inflict many bites if not handled with extreme care.

The results of this study supports the conclusions of Chitty (1952) that more severe stress can be transmitted to the next generation, and of Christian and LeMunyan (1958) that such suppression of progeny can persist for at least two generations due to degradation of either quality or quantity of milk.

Thus, such stress factors as overcrowding, cold, and nutritional deficiency proved repeatedly to cause population declines. These factors can also delay the build-up of numbers after the crash, even if the stress factors are completely eliminated. In addition to the low survival of young produced by the stressed mothers, another delaying factor, not previously observed, became evident in this study; namely, long delayed maturity and widely spaced litters. A high proportion of the  $F_1$  generation females never produced young, and those that did reproduce averaged five months of age at the time of parturition. At least half of these mothers never produced another litter, and those that did produce additional litters

averaged over two months between litters. Such reduced rate of reproduction, coupled with slow maturing and low survival provide an explanation for the slow recovery of a population after a crash. As both Chitty (1952) and Christian and LeMunyan (1958) observed, the effects on the young can be explained, at present, only on the basis of inadequate lactation on the part of the stressed mother, in turn putting a severe stress (of starvation) on the nursing young. Continuing retardation of the second generation of young must result from failure of recovery from the stresses suffered during lactation. Whether such prolonged effects are results from adrenal exhaustion, impairment of pituitary function, or to general debilitation can only be surmised at the present time.

Before an understanding of the total effect of stress factors on a population can be attained, much additional work will be necessary. In an attempt to find levels of stress and the limits the animal can withstand, mice must be fed different levels of protein. Further effects on the females can be checked in  $F_1$  females by trading litters, which can be done successfully on the third day of lactation. Litters of controls and experimentals could be traded, if born the same day, to see if the limiting factor is the quality or quantity of milk. Litters could be reduced to further test whether the factor is actually the quality or quantity. Analyzing milk would be of importance, although other workers seem to disregard quality of milk as an important factor.

The reproductive capacity of affected females could be tested by injections of gonadotropins, and prolactin could be administered to determine whether the limiting factor for lactation is a pituitary function.

Interrelationships between the environment and a population comprise an extremely complex situation, and many factors must be considered before a complete understanding of relationships can be expected.

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RATE OF LITTER SURVIVAL FROM STRESSED  
MOTHERS THROUGH THE F<sub>3</sub> GENERATION

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AN ABSTRACT OF A THESIS

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This study was undertaken to determine (1) effects of stress on development of young, (2) different stress factors and their relationships, (3) levels of stress capable of causing detrimental effects without completely destroying the young, and (4) effects of stress that may be transmitted to the young, and how long these effects might last.

A control and three experimental groups of 15-30 white mice each were used. Group I (control) females were treated in a manner allowing maximum reproduction and growth. Group II (cold group) was treated as the controls except they were maintained at 2-3° C isolated in wire cages with no nesting materials for one hour a day, from occurrence of a copulation plug until weaning. Group III (cold after parturition) was placed in the cold at 2-3° C for one hour a day from the day of parturition until weaning. Group IV (low protein) was maintained on a diet consisting of equal parts corn meal and corn starch with mineral and vitamin additives from the time of parturition until weaning. Experimental procedures were administered only to the parental generation. All offspring were treated the same as the controls through all subsequent generations. A low water group was attempted but failed because of extreme individual differences in water requirements.

The criteria used to determine effects of treatment were: numbers and weights of suckling young at birth, 5, 10, 15 and 20 days after birth; weight at 60 days; age at parturition, and reproductive success.

The individual weights of F-1 generation young of all experimental groups except Group II were significantly lower than those of the controls; Group I, 8.8 gm; Group II, 8.8 gm; Group III, 7.8 gm, and Group IV, 5.1 gm. All experimental groups showed a higher mortality rate, greatest in the low

protein group; survival was 66 percent in Group I, 42 percent in Group II, 64 percent in Group III, and 35 percent in Group IV. Group II had a high percentage of resorbed embryos, but other effects were not as severe as in other groups.

The average weight of individuals in F-2 young was 8.6 gm in Groups I and II, 7.3 gm in Group III, and 8 gm in Group IV; all significantly lower ( $p < 0.05$ ) except for Group II. In Group IV, age at maturity was most highly affected, with nearly six months passing before an appreciable number of the F-1 group reproduced and four months before the F-2 group reproduced. Only 10 percent of the F-1 Group IV produced litters by six months, and 40 percent of the F-2 littered by four months, compared to 70 percent of the control F-1 by three months.

The third generation in all groups returned to normal.

It was concluded that severe stress applied to parents could affect offspring to the third generation. The more severe the stress, the greater and more long lasting was the effect on the young. Animals severely affected produced only two-thirds as many litters as did non-affected animals causing declines in total population growth.